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Behavior, welfare, production and bioenergetics of laying hens in alternative housing systems

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Behavior, welfare, production and bioenergetics of laying hens in alternative housing systems

by

Jofran Luiz de Oliveira

A dissertation submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major: Agricultural and Biosystems Engineering

Program of Study Committee:
Hongwei Xin, Co-major Professor
Steven James Hoff, Co-major Professor
Suzanne Theresa Millman
Huaiqing Wu
Lie Tang

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this dissertation. The Graduate College will ensure this dissertation is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2019

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DEDICATION

To my wife *Maria*, who bravely embraced me and my dreams in this epic journey.

To my daughter *Laura*, who empowered me with her curiosity and endless questions about everything. May you never stop questioning.

To *science*, that amazingly improved my education.

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ABSTRACT

Transitioning of egg production systems from conventional cage to alternative housing (e.g., enriched colony, aviary cage-free) is increasingly occurring in various parts of the world, especially in Europe and the United States, to meet animal welfare requirements or legislations. This dissertation had the central goal of providing scientific knowledge or discoveries related to behavior, welfare, production, and bioenergetics of laying hens in alternative housing systems. It covers five experiments that were conducted in controlled environment and commercial settings with the following specific objectives: 1) Develop and validate a UHF RFID system able to evaluate nesting and feeding behaviors of laying hens in an Enriched Colony Housing (ECH) (Chapter 2); 2) Evaluate the impact of feeder space on feeding behavior of individual hens in an ECH (Chapter 3); 3) Investigate nesting behavior and nesting patterns of individual hens in an ECH (Chapter 4); 4) Assess the impact of managing litter floor access and using experienced hens on floor eggs, air quality and welfare of hens in an aviary system (Chapter 5); and 5) Quantify building ventilation rate (VR) and laying-hen bioenergetics in a fully-open aviary house (Chapter 6).

The research described in this dissertation contain the following discoveries: The UHF RFID system was successfully developed and validated. The system allows for assessing the impacts of housing design and/or management practices on behaviors of individual laying hens (Chapter 2). Laying hens (W-36 breed) in the ECH showed similar feeding behaviors when provided a feeder space of 12 or 9.5 cm/hen, and not all hens choose to feed simultaneously (Chapter 3). Hens spent approximately one hour in the nest box during a 16-hr daily light period. However, nesting time during the 6-hr laying period

(37.5% of the light period) accounted for 56% of the daily total. Maximum occupancy of the nest box (29% of the hens) occurred within 4-hr after lights-on, when most (83%) of the eggs were laid. There exist considerable hen-to-hen variations in nesting behavior. The same is true for an individual hen from one day to the next, although specific patterns could be noted (Chapter 4). Full litter access (FLA) in the aviary housing system showed a number of shortcomings when compared with part-time litter access (PLA), including much higher incidence of floor eggs, higher ammonia concentration, more presence of caked litter, and greater amount of manure accumulation on the floor which necessitates more frequent removal from the barn. No difference was detected between FLA and PLA in hen welfare, mortality, BW, BW uniformity, or litter bacteria concentration. Inclusion of experienced hens (1.5%) in a young flock did not show benefit of inducing nest-laying behavior (Chapter 5). Mean ventilation rate (VR) of a fully-open aviary house (~ 140,000 Dekalb White laying hens) under the Midwest (Iowa) climate conditions (outside temperature ranging from 3.4 to 28.9°C) was 4.0 ± 0.4 $m^3\ h^{-1}\ hen^{-1}$, ranging from 0.8 to 9.1 $m^3\ h^{-1}\ hen^{-1}$. Overall, daily mean total heat production rate (THP) was 7.5 ± 0.2 W kg^{-1}, house-level sensible heat production rate (SHP) was 4.8 ± 0.3 W kg^{-1} and house-level latent heat production rate (LHP) was 2.7 ± 0.2 W kg^{-1}. THP decreased by 40% in the nighttime or dark period (5.1 ± 0.3 W kg^{-1}) as compared to the daytime or light period (8.5 ± 0.3 W kg^{-1}). Information from this dissertation research is expected to contribute to establishment or improvement of guidelines for housing system design and management to ensure animal welfare and efficient use of resources in alternative laying-hen housing. In particular, the updated bioenergetics data will prove valuable in estimating building ventilation rate using the indirect calorimetry or carbon dioxide (CO_2) balance method, and improving the design and operation of ventilation, supplemental heating, and cooling components of the housing.

CHAPTER 1. GENERAL INTRODUCTION

This chapter provides a knowledge basis and foundation for the chapters that follow, defining the issues this dissertation was set out to address, and justifying the potential contributions the research is to make to the field. Accordingly, the objectives and structure of the dissertation are articulated.

Sustainability of alternative housing systems

Sustainability is often related to environmental impact, but the concept of sustainability in animal agriculture is also associated with a belief that in the long term the sustainable activity must be able to incorporate the following key aspects: food affordability, food safety, worker safety, environmental impact, and animal welfare. The challenge of sustainability in the current animal production system, especially activities related to the egg industry, is to balance the animal welfare against the other sustainability keys (Mench, 2018).

To address the lack of scientific information on the sustainability aspects of the egg supply, a group of organizations (Coalition for Sustainable Egg Supply – CSES) developed a series of research performed on commercial farms in the Midwestern United States. Several variables of the sustainability keys were evaluated over a three-year period with two flocks of hens in three different types of laying hen housing (conventional, enriched colony and cage-free aviary). Results from this coalition study are currently used, in some instances, to guide production and support decision-making.

Welfare in the field of animal production is a term used to express ethical concerns about the quality of life of those animals. It is established as science, but there is no precise scientific definition on animal welfare (Duncan, 2005). There are three approaches to assess welfare emphasizing characteristics such as affective (e.g., pleasure and suffering), biological (e.g., disease and injury) and nature (e.g., ability to express natural behavior). The idea that the animal should be allowed to express natural behaviors in a natural environment is difficult to apply to the animal production system but can be translated as respecting the animal nature identifying important factors and meeting its needs as far as possible (Appleby et al., 2004).

The hens' welfare status is usually assessed on the farm by the welfare quality protocol (Welfare Quality®, 2009), which focuses more on animal-based measures and comprises of both physical and behavioral measures listed in Table 1.1.

To facilitate decision making it is essential to understand the associations of the housing design and the implications on animal welfare and food affordability. Zhao et al. (2015) presented a comparison on the housing design of the three different styles of laying hen housing evaluated in the CSES study: Conventional Cage, Enriched Cage, and Aviary Cage-free.

The conventional or battery cage

The laying hens are raised in small wire cages, in groups of 5-10 hens with access to water and feed (Figure 1.1).

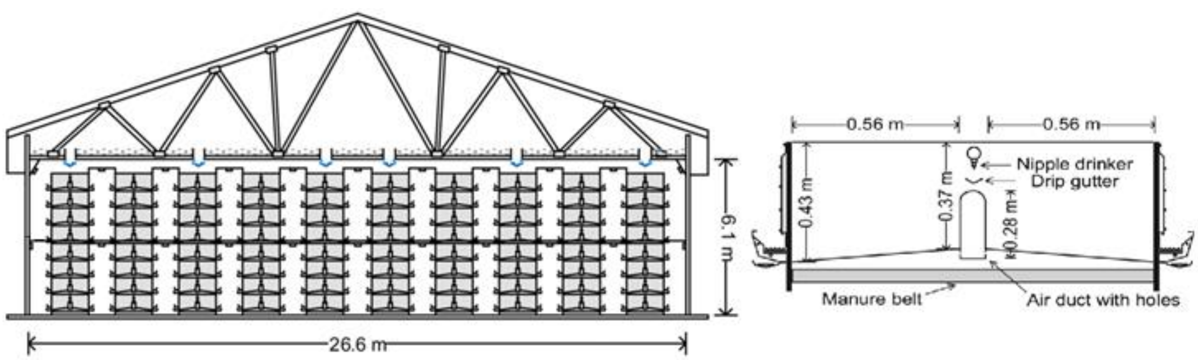

Figure 1.1 *Schematic layout of the conventional house and conventional cage system. Adapted from Zhao et al. (2015).*

Table 1.1 *Measures of welfare assessment of laying hens on farm based on the Welfare Quality® protocol (Welfare Quality®, 2009).*

	Welfare criteria		Measures
Good feeding	1	Absence of prolonged hunger	Feeder space
	2	Absence of prolonged thirst	Drinker space
Good housing	3	Comfort around resting	Shape and total length of available perches, evidence of red mites dust sheet test
	4	Thermal comfort	Panting, huddling
	5	Ease of movement	Stocking density, perforated floors
Good health	6	Absence of injuries	Keel bone deformation, skin lesions, foot pad dermatitis, toe damage
	7	Absence of disease	On farm mortality, culls on farm, enlarged crops, eye pathologies, respiratory infections, enteritis, parasites, comb abnormalities
	8	Absence of pain induced by management procedures	Beak trimming
Appropriate behavior	9	Expression of social behaviors	Aggressive behavior, plumage damage, comb pecking wounds
	10	Expression of other behaviors	Use of nest boxes, use of litter, enrichment measures, free range, cover on the range, covered veranda
	11	Good human-animal relationship	Avoidance distance test (ADT)
	12	Positive emotional state	Novel object test (NOT), qualitative behavior assessment (QBA)

The enriched colony

The laying hens are raised in larger cages than in conventional systems, in groups of 60-100 hens, with access to water, feed, perches, colony nests and scratch pad (Figure 1.2).

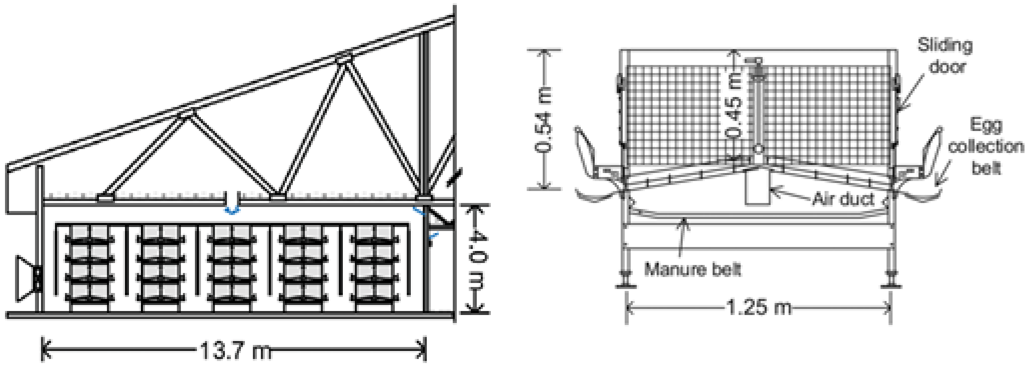

Figure 1.2 *Schematic layout of the enriched colony house and enriched colony system. Adapted from Zhao et al. (2015).*

Compared to conventional systems, the total cost per dozen eggs in the enriched colony housing is 13% higher. It stems from higher costs on labor (similar labor force but fewer hens housed) and capital (costs associated with the barn construction and the relatively few hens housed). Costs on feed and pullets are lower than in the conventional production (affected by mortality, production and feed conversion). The costs of energy in enriched and conventional cages are similar (Matthews and Sumner, 2015).

The cage-free (Aviary)

The laying hens are raised free from cages, with hens grouped in thousands, and access to water, feed, perches, colony nests, litter area for foraging and dust bathing (Figure 1.3).

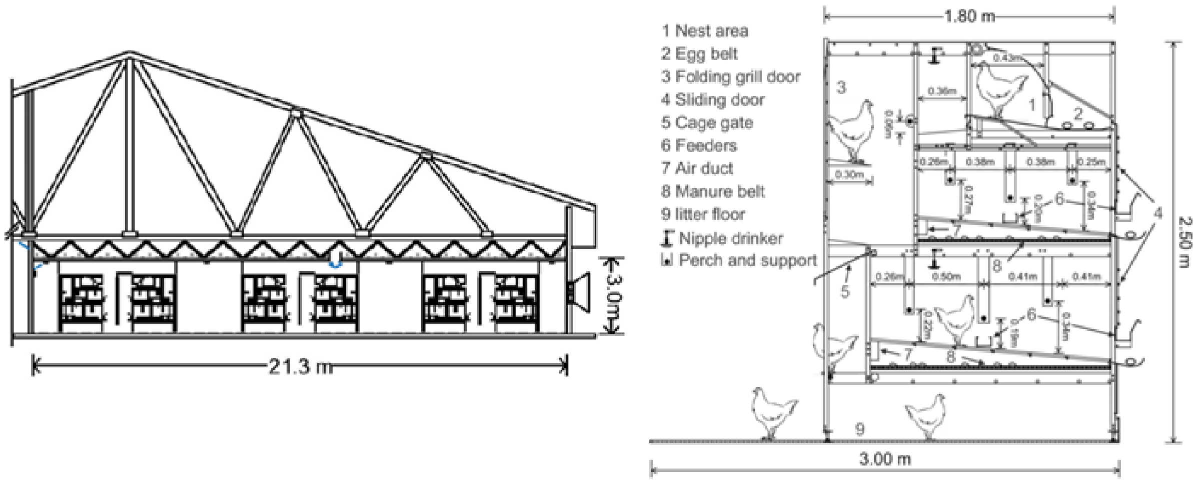

Figure 1.3 *Schematic layout of the cage-free aviary house and aviary system. Adapted from Zhao et al. (2015).*

Compared to conventional systems, the total cost per dozen eggs in the aviary house (of the CSES study) is 36% higher. It stemms from higher costs on labor (more labor needed and fewer hens housed), feed (similar feed consumption but less productivity at the end of cycle due to higher mortality), pullet (higher rearing cost and lower livability), and capital (costs associated with the barn construction and the relatively fewer hens housed). The costs of energy in the aviary system and conventional cages are similar (Matthews and Sumner, 2015).

Based on the CSES and other literature, measures on animal welfare are presented in a comparison table (Table 1.2). For better understanding/visualization, the cells were colored green, yellow and red to indicate good, moderate and poor welfare condition, respectively.

Table 1.2 *Comparative on animal welfare among housing designs.*

WELFARE VARIABLES	HOUSING DESIGN			REFERENCES
	Conventional	**Enriched**	**Cage free**	
Mortality	Well controlled in a system under proper management. Cumulative mortality of ~4.7%	Mortality in enriched cages is comparable to that in a conventional system. Cumulative mortality of ~4.9%	Highest mortality among the systems, with many diseases related to soil/litter contact and dust exposure. Cumulative mortality of ~11.7%	(Lay et al., 2011)
Behavior	Hens are completely restricted to perform certain natural behaviors (flying, running, foraging, dust bathing, nesting, and perching). Hens are kept in small social groups.	Improvement of cage design allows hens to perch, scratch and lay the eggs in colony nests. Hens have more space (lower stocking density) and bigger social groups.	Hens have access to litter area and sometimes outdoor access (free range). Hens are allowed to fly and perform dust bathing. Hens' social groups are the largest in this system.	(Lay et al., 2011)
Cannibalism/ aggression	Low incidence of cannibalism, feather pecking, and social aggression.	Moderate incidence of cannibalism and feather pecking when the hens are housed in larger groups. Moderate occurrence of social aggression.	High incidence of cannibalism and feather pecking. However, low occurrence of social aggression.	(Lay et al., 2011; Blatchford et al., 2016)
Keel damage	Low occurrence of keel bone deformation overall.	Moderate prevalence of keel damage, presumably because of perching.	High prevalence of keel damage. Besides perching, hens in this systems experience direct impact of the keel bone during short flight.	(Lay et al., 2011)
Bone strength	Due to movement limitation, hens housed in conventional cages presented the worst conditions of bone strength.	Improved bone strength when compared to the conventional system, presumably due to perching and scratching activities.	Best conditions for bone strength. Hens are able to perform extra physical activities such as foraging, flight and running, which can contribute to bone strength.	(Tactacan et al., 2009; Regmi et al., 2016; Yilmaz Dikmen et al., 2016)
Feather condition	Feather loss is due more to cage wear	Feather loss is due more to cage wear	Feather loss more due to pecking	(Tactacan et al., 2009; Blatchford et al., 2016; Yilmaz Dikmen et al., 2016)

Challenges for egg production in alternative housing systems

The egg production sector has changed considerably during the past decade, especially in terms of stocking density and housing design with an increased availability of enrichments (perches, scratch areas, nest boxes, litter area) which aims to accommodate natural expression of animal behaviors and consequently enhancement of welfare. The CSES Study has progressed toward establishment of baseline information that holistically shows the advantages and disadvantages of housing designs (Conventional, Enriched Colony and Aviary) regarding several aspects of egg production sustainability. However, some points remain unclear and still challenge the egg industry, as outlined below.

1) Provision of proper feeder space is a crucial parameter in hen housing design. However, feeder space guidelines from hen welfare standpoint vary considerably among and within countries. To achieve and apply consistent guidelines and, more importantly, to ensure the provision of proper feeder space to satisfy the animal's needs while maximizing the efficiency of resource utilization, the following fundamental questions need to be answered: *What feeder space do the hens need? How much time do they usually spend at the feeder each day? How frequently do they use the feeder? Do they opt to feed at the same time?* It is expected that answers to such questions would depend on the type of housing system and the level of housing enrichment.

2) Although some studies have been conducted to evaluate animal behaviors with the aid of RFID, information regarding nesting behaviors of laying hens in enriched colony housing (ECH) is lacking, and the following questions remain further addressed: *How much time do the hens usually spend inside the nest box each day? How frequently do they use the nest box? What is the maximum simultaneous occupancy of hens in the*

nest box and how it changes during the day? Such information has implications on the design and allocation of enrichment and management practices.

3) There is a general concern that limiting litter access of hens would affect expression of the animal's natural behaviors, which may lead to compromised welfare. There has also been an anecdotal claim that confining hens inside the systems negatively affect flock uniformity. However, data are lacking to substantiate the concerns or claims and answer the following questions: *Does managing litter access affect animal welfare? Does it reduce the number of floor eggs? Could hens trained in using nest boxes teach pullets and thus reduce the number of floor eggs? What are the impacts of managing access to litter area on litter condition and air quality?* Answers of these question can influence the farms' management practices and consequently their productivity and environmental impact.
4) The change of stocking density and addition of enrichments in the alternative housing systems contribute to the increase in hen activity, consequently changing their heat production. Studies on bioenergetics of laying hens have been performed using calorimetric chambers housing conventional cages or aviary unit, and certain type of whole-house commercial aviary. However, in modern fully-open cage free production facilities, nominal housing capacity has increased from 50,000 to as many as 140,000 hens in one room. Therefore, the following questions need answered: *How much heat does a chicken produce in a fully-open modern aviary facility? What is the circadian or diurnal pattern of the heat production? How is the total metabolic heat production partitioned into sensible and latent mode at the house level?* Such updated house-level heat production data will prove valuable for indirectly calculating building ventilation

rate (i.e., through carbon dioxide balance method), and improving design and operation of the ventilation, supplemental heating and cooling systems.

Organization of the Dissertation and Objectives

The dissertation is divided into seven chapters, where chapter 1 covers the general introduction, the chapters 2 to 6 report the experiments with the specific objectives of this dissertation, and finally the chapter 7 summarizes the dissertation's conclusion. To address some of the challenges and to fill some of the knowledge gaps towards achieving sustainability of alternative housings systems, the experiments described in this dissertation aim to accomplish the following specific objectives:

1) Design, develop, evaluate, and apply a UHF RFID automated monitoring system for characterization of feeding and nesting behaviors of individual hens in a commercial enriched colony housing (ECH) module (Chapter 2);
2) Evaluate the impact of feeder space on feeding behaviors of laying hens in an ECH, focusing on (1) daily time spent (TS, min/hen-day) at the feeder, (2) daily frequency of feeder visits (FV, #/hen-day), and (3) synchronization of feeding as measured by maximum and average percentage of hens feeding simultaneously (MPB and APB, %). Quantify the variability in feeding behaviors (TS and FV) among the individual hens. Assess the effect of feeder space on production performance as measured in group-average daily feed intake (FI, g/hen-day), water use (WU, g/hen-day), and hen-day egg production (HDEP, %). (Chapter 3);
3) Characterize nesting behavior of W36 laying hens with the aid of the UHF RFID system, including: daily time spent in the nest box (TS, min/hen-d), daily frequency of visits to the nest box (FV, visits/hen-d), number of visits per egg laid in the nest box

(VE, visits/egg), simultaneous occupancy of the nest box (SO, %), oviposition time (OT, hh:mm), and oviposition place (OP, % eggs laid in the nest, middle or scratch areas), and nesting association network (Chapter 4);

4) Evaluate the effects of full litter access (FLA) vs. part-time litter access (PLA) and the inclusion of experienced hens or not on the occurrence of floor eggs, litter condition, indoor air quality, and hen welfare through a long-term field study involving a commercial aviary hen housing system (Chapter 5); and
5) Determine ventilation rate and quantify house-level heat production (THP, SHP, and LHP) of Dekalb White laying hens in a modern commercial fully-open laying hen aviary house (Chapter 6).

Expected Outcomes and Practical Implications

The experiments presented in this dissertation are expected to fulfill some of the scientific knowledge gaps that help address the challenge toward egg production sustainability. Specifically, this research will (1) to demonstrate new technologies/methods used to evaluate feeding and nesting behavior of individual hens kept in groups, (2) to update information on laying hens heat production (house-level) so the future ventilation design can be done more efficiently, and (3) to support decision-making with regard to management of litter access. Findings from this dissertation will support the egg industry and regulatory agencies in making more informed, science-based decisions toward modifying production practices or designing ventilation systems for a more efficient utilization of resources.

References

Appleby, M. C., J. A. Mench, and B. O. Hughes. 2004. Poultry Behavior and Welfare. CABI Publishing, Wallingford, Oxfordshire, UK, Cambridge, MA USA.

Blatchford, R. A., R. M. Fulton, and J. A. Mench. 2016. The utilization of the Welfare Quality ® assessment for determining laying hen condition across three housing systems. Poult. Sci. 95:154–163.

Duncan, I. J. H. 2005. Science-based assessment of animal welfare: farm animals. Rev. Sci. Tech. – Off. Int. des Epizoot. 24:483–492.

Lay, D. C., R. M. Fulton, P. Y. Hester, D. M. Karcher, J. B. Kjaer, J. A. Mench, B. A. Mullens, R. C. Newberry, C. J. Nicol, N. P. O'Sullivan, and R. E. Porter. 2011. Hen welfare in different housing systems. Poult. Sci.

Matthews, W. A., and D. A. Sumner. 2015. Effects of housing system on the costs of commercial egg production. Poult. Sci. 94:552–557.

Mench, J. A. 2018. Advances in poultry welfare. Elsevier.

Regmi, P., N. Smith, N. Nelson, R. C. Haut, M. W. Orth, and D. M. Karcher. 2016. Housing conditions alter properties of the tibia and humerus during the laying phase in Lohmann white Leghorn hens. Poult. Sci. 95:198–206.

Tactacan, G. B., W. Guenter, N. J. Lewis, J. C. Rodriguez-Lecompte, and J. D. House. 2009. Performance and welfare of laying hens in conventional and enriched cages. Poult. Sci. 88:698–707.

Welfare Quality ®. 2009. Welfare Quality ® Assessment protocol for poultry. Lelystad, Netherlands.

Yilmaz Dikmen, B., A. İpek, Ü. Şahan, M. Petek, and A. Sözcü. 2016. Egg production and welfare of laying hens kept in different housing systems (conventional, enriched cage, and free range). Poult. Sci. 95:1564–1572.

Zhao, Y., T. A. Shepherd, J. C. Swanson, J. A. Mench, D. M. Karcher, and H. Xin. 2015. Comparative evaluation of three egg production systems: Housing characteristics and management practices. Poult. Sci. 94:475–484.

CHAPTER 2. A UHF RFID SYSTEM FOR STUDYING INDIVIDUAL FEEDING AND NESTING BEHAVIORS OF GROUP-HOUSED LAYING HENS

L. Li, Y. Zhao, J. Oliveira, W. Verhoijsen, K. Liu, H. Xin

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Abstract

Enriched colony housing (ECH) is a relatively new egg production system. As such, information is lacking on design parameters to ensure the well-being of the hens and optimal utilization of housing resources. A new system has been developed at Iowa State University that enables automated monitoring and quantification of feeding and nesting behaviors of individual hens in ECH. Ultra-high-frequency radio frequency identification (UHF RFID) is employed to track individual animals. The UHF RFID system consists of four components: antennas, tags, readers, and a data acquisition system. The antennas for monitoring feeding behavior are placed inside the two feed troughs and covered with plastic boards. Each feed trough has six antennas aligned in series covering the length of the feeder. Four additional antennas are placed inside the nest boxes to monitor the nesting behaviors. All 16 antennas are connected to five 4-channel readers, two per feed trough and one for the nest boxes, that are further connected to the hosting computer via Ethernet. Feed and water consumption and egg production are continuously monitored using load cells. This article describes the development and testing of the RFID system for monitoring feeding and nesting behaviors and provides sample data. The system has proven to be able to characterize benchmark feeding and nesting behaviors of individual hens in ECH, such as daily time spent at the feeder and in the nest box, daily frequency of visiting the feeder and the nest box, number of hens feeding and nesting simultaneously, and variability in these behaviors among individual hens. Future applications of the system include assessing the impact of resource allocation and management practices on feeding and nesting behaviors and on the well-being of the hens. This information will provide a scientific basis for optimal design and management of alternative hen housing systems.

Keywords. Animal well-being, Enriched colony housing, Feeding behavior, Nesting behavior, UHF RFID.

Introduction

Enriched colony housing (ECH) systems for laying hens originated in the 1980s in Europe and were intended to improve animal welfare by allowing the birds more opportunity to express their natural behaviors. Compared with conventional cage housing (CC), ECH features larger space allocation (e.g., 748 vs. 432 cm^2 per hen), larger group size (e.g., 60 hens per colony vs. 6 to 8 hens per cage), as well as perches, scratching pads, and nesting boxes designed to allow laying hens to express natural behaviors (Mench et al., 2011; Zhao et al., 2015). Relative to CC, which has been used for more than half a century, much less is known about the optimal design and operation of ECH. For instance, different opinions and guidelines exist regarding feeder space and nest area requirements for ECH. Some studies call for provision of enough feeder space to accommodate all hens in the colony simultaneously and a minimum of 300 cm^2 of nest space (Appleby, 2004; Bracke and Hopster, 2006; UEP, 2016). The feeder and nest space requirements have a significant impact on the design and management of housing systems. For instance, to allow all hens to feed at the same time, extra feed troughs would be necessary, which can complicate both the housing structure and day-to-day production management. To address these critical questions, methods for quantifying the behavioral and performance responses of animals, especially individual animals in groups, to these design and management factors are imperative (Ben Sassi et al., 2016; Tu et al., 2011).

Animal behavior studies commonly rely on direct visual observation or videotaping, followed by manual analysis. These manual approaches are inevitably laborious, time-demanding, and prone to errors because of subjective interpretation or inattention (Catarinucci et al., 2014). With increasing emphasis on precision animal farming and monitoring of individual animals on a continuous basis, manual approaches will be a thing of the past. Automated monitoring systems for assessment of animal behavior and well-being, whether for research or in commercial production, will be the norm in the future.

One automated technology is radio-frequency identification (RFID), which uses electromagnetic fields to identify tags attached to objects. This technology has been broadly applied to behavioral monitoring of animals (Brown-Brandl and Eigenberg, 2011; Cappai et al., 2014; Maselyne et al., 2014a, 2014b; Nakarmi et al., 2014; Sales et al., 2015; Samad et al., 2010; Tu et al., 2011). Vouldimos et al. (2010) developed a complete farm management system based on animal identification using RFID technology with mobile wireless networking to track animals and create a repository of animal data records. RFID devices have also been applied to quantify some behavioral traits of laying hens. Nakarmi et al. (2014) developed a novel low-frequency (LF) RFID system for automated quantification of locomotion, perching, feeding, drinking, and nesting behaviors of individual laying hens in a group-housed setting. While this system produced satisfactory behavioral data for individual hens, it worked only for small animal groups of no more than ten hens due to the limitation of LF RFID with relatively slow data-transfer speed. For larger animal groups, faster data-transfer systems are needed to read more animal tags in a short time.

The objective of this research was to design, develop, evaluate, and apply a UHF RFID automated monitoring system for characterization of feeding and nesting behaviors of 60 individual hens in a commercial ECH module. Accuracy of the RFID system was validated through manual labeling of visual observation data.

Materials and Methods

Experimental Hen Room and Enriched Colony Housing (ECH) Module

The experimental room had the dimensions of 11.4 m long × 6.6 m wide × 4.3 m high (figs. 2.1 and 2.2). It housed two double-tier ECH modules (Big Dutchman, Inc., Holland, Mich.), with room for two more modules if needed. Two variable-speed exhaust fans (max. airflow rate of 1500 m^3 h^{-1} each) were installed in the back wall to create a negative static pressure in the room. Two perforated intake air ducts were designed to achieve uniform air distribution of the ventilation air, following the instruction of Harmon (2008). Three pairs of programmable LED lights were used to provide the lighting. The two lights of each pair were hung at heights of 1.0 and 1.7 m, respectively, above the floor to light the top and bottom tier colonies.

The double-tier ECH module was 3.73 m long × 1.91 m wide × 1.91 m high (fig. 2.3). The colony in each tier was equipped with perches, nest boxes, and scratch pads. Feed troughs were located on both sides of the colony. Manure was collected on a plastic tarp placed underneath the colony tier and was removed weekly or more often. The normal capacity of the colony was 60 hens. Table 2.1 shows the resource allowance of the ECH module.

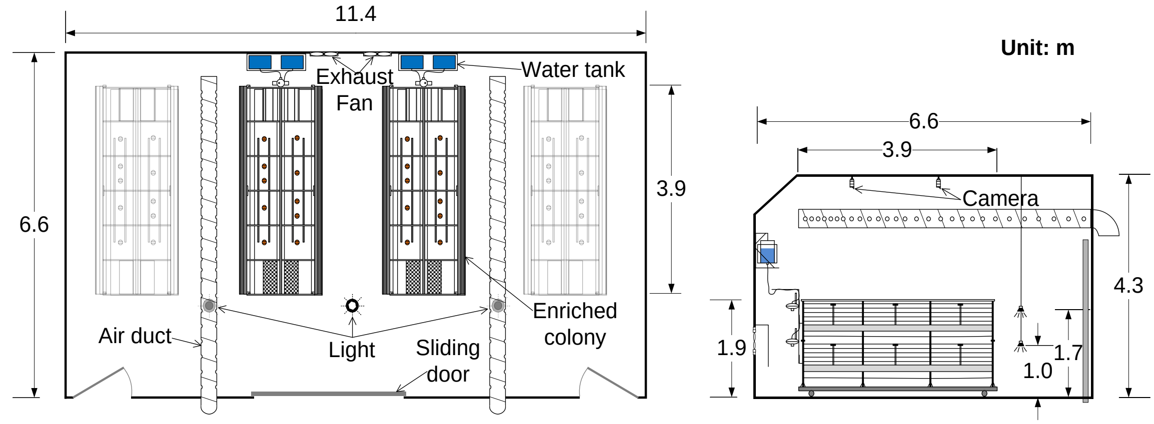


Figure 2.1 *Top view (left) and side view (right) schematic drawings of the experimental hen room.*

Figure 2.2 *Photograph of the experimental hen room with enriched colony housing (ECH) modules.*

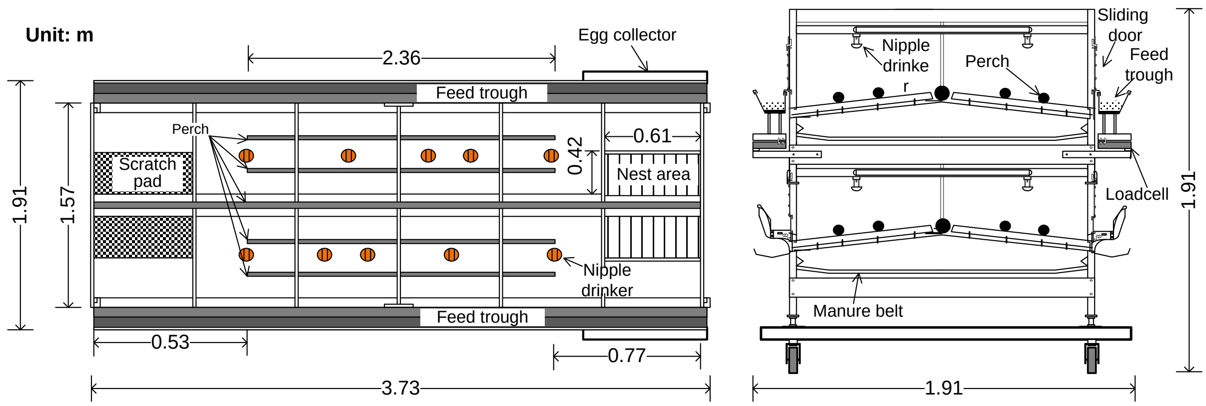


Figure 2.3 *Top view (left) and side view (right) schematic drawings of the enriched colony housing (ECH) module.*

Table 2.1 *Resource allowance of enriched colony (60 hens per colony).*

Resource	Unit	Allowance
Floor area	cm^2 hen^{-1}	976
Nest box area	cm^2 hen^{-1}	85.4
Nipple drinker	hens $drinker^{-1}$	10
Feeder space	cm hen^{-1}	12.3
Perch length	cm hen^{-1}	15.7

The top-tier colony was instrumented to monitor real-time feed and water use and record egg production (timing and number) via load-cell scales, and to measure the feeding and nesting behaviors of individual hens via the UHF RFID system. Load-cell scales (Rice Lake Weighing Systems, 0 to 30 kg, Rice Lake, Wisc.) were used to continuously (every second) weigh the feeders, the water tank, and the egg collector (fig. 2.4). The outputs of the individual load-cell scales for each feed trough were combined to obtain the total weight of the feeder.

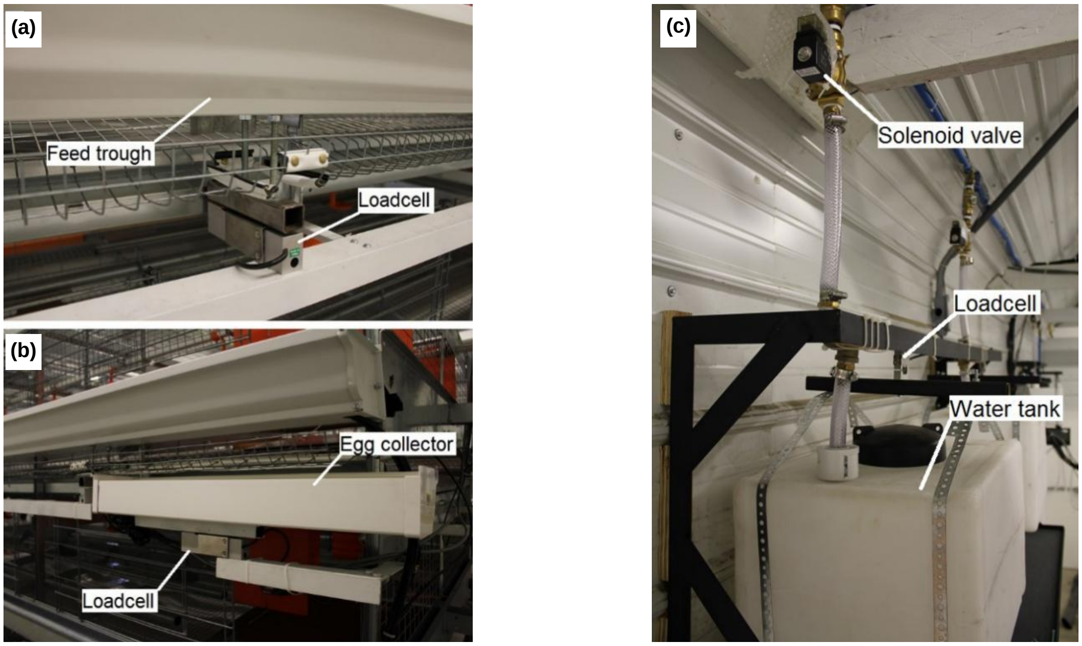


Figure 2.4 *Weighing systems for (a) feed trough, (b) egg collector, and (c) water tank.*

Air temperature was measured with four thermocouples, two for the hen room, one for the control room (where the DAQ system was located), and one for outdoor. Relative humidity (RH) measurement of the hen room, collocated with the temperature sensors, was made with RH sensors (model HMT100, Vaisala, Inc., Woburn, Mass.). Carbon dioxide (CO_2) levels at the two locations of the hen room were measured with Vaisala GMT222 CO_2 sensors. The temperature, RH, and CO_2 sensors were regularly checked and calibrated, as necessary. All sensors were connected to a compact FieldPoint module, and the output signals were recorded using a LabVIEW program (National Instruments, Austin, Tex.).

The UHF RFID System

The UHF RFID system (TransTech Systems, Aurora, Ore.) consists of four elements: antennas, tags, readers, and a data acquisition (DAQ) system. Each antenna generates an electromagnetic field that automatically registers the tags within its field (detection range) and sends the tag ID to the DAQ through a reader.

Antennas for the feeders were placed at the bottom of the feed troughs and covered with plastic boards (fig. 2.5). Each feed trough had six antennas (four SlimLine 8060, 864 to 869 MHz and 902 to 928 MHz, 65 cm long × 8.6 cm wide × 0.8 cm thick each; and two IPJ-A0311-USA, 46 cm long × 8.9 cm wide × 1.9 cm thick each, TransTech Systems, Aurora, Ore.) aligned in series and covering the length of the feeder. The antennas were situated such that the tag (902 to 928 MHz, PT-103, tie-wrap tag passive Gen 2 UHF, TransTech Systems) attached to a hen's neck could be registered when the hen was present at the feeder. Antennas (Square A1030, 30 cm × 30 cm × 0.65 cm thick) for monitoring the nesting behaviors were placed underneath the nest box mats, two antennas per nest box. All 16 antennas (12 for feeders and 4 for nest boxes) were connected to five 4-channel readers (ThingMagic Mercury M6, 865 to 928 MHz operating frequency, TransTech Systems) that were further connected to the computer via an Ethernet connection (fig. 2.6).

The readers were connected to a host computer that processed the tag data through an RJ45 (Registered Jack, 10/100 Base-T Ethernet). The tag protocol was EPC global Gen2 (ISO 18000-6C) with digital rights management (DRM). The data acquisition program was written in C# (C Sharp) based on an application programming interface (API), and the data were stored as text files. Data analysis and processing were realized using the SQL server and Excel VBA programs.

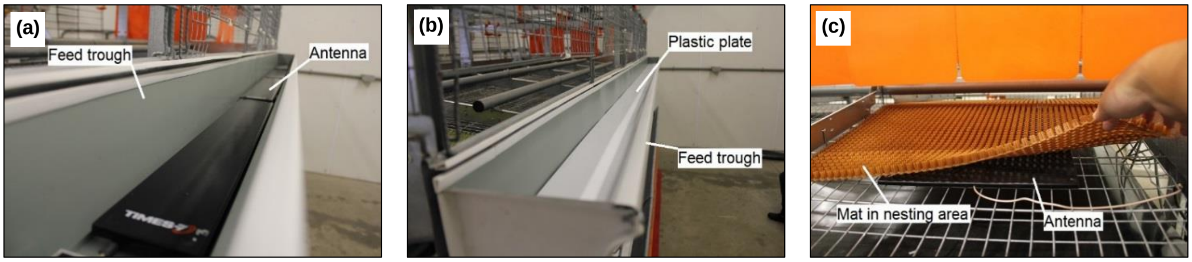


Figure 2.5 *RFID antennas (a) placed in the feed trough and (b) covered with plastic plates, and (c) the nest box antenna covered with mat.*

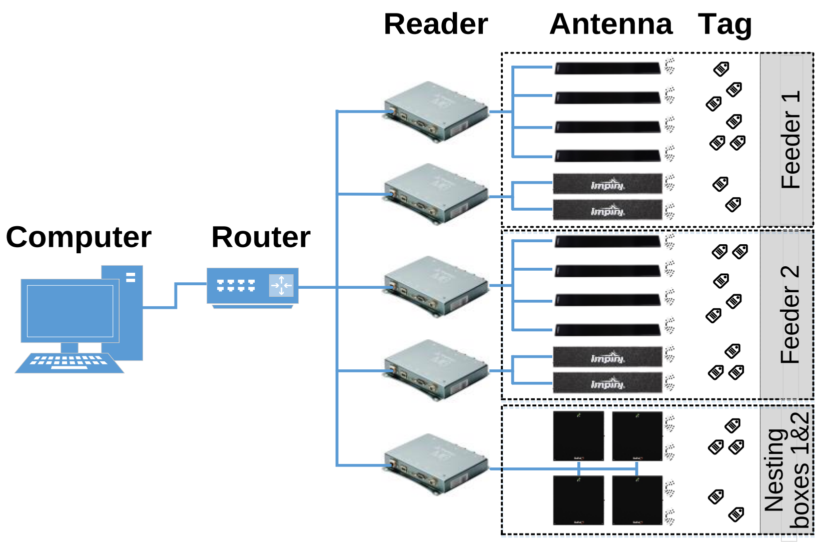


Figure 2.6 *Interfacing of the RFID system components.*

Video Observation and Processing

Two cameras (IP Pro 3 Megapixel Bullet, DSS-BFR3MP, Backstreet Surveillance, Salt Lake City, Utah) were installed on the ceiling above the ECH module and used to record the hen behaviors at 2 frames per second (fps). The two cameras were wired to an 8-port power-over-Ethernet (POE-108, Backstreet Surveillance) injector. Video files were stored in 8 terabyte storage (two hard drives) of an NVR system (DSS-NVR5816, Backstreet Surveillance). Each camera covered half of the colony area. Images taken by the two cameras at the same time stamp were extracted from the video files, corrected for distortion, and stitched into one image using a program developed in Matlab (R2015b, MathWorks, Natick, Mass.).

System Performance Tests

Calibration of Load Cells

Egg production, feed consumption, and water consumption were continuously (every second) monitored by the load-cell scales mounted under the egg collector, under the feeders, and above the water tanks. The weights of the egg collectors and water tanks were monitored individually with one calibrated load cell. Each feeder had three scales that were calibrated separately and collectively to develop individual standard curves (by separate calibration) and one combined curve (by collective calculation). Individual and combined standard curves were developed using four weights (1600 g each) that created five load levels (0, 1600, 3200, 4800, and 6400 g). At each load, signals (in V) from the load cells were read every second for 10 to 20 s following stabilization. These signals were averaged and regressed to the corresponding load levels to develop the standard curves.

Measurement accuracy of the overall feed weight in a feeder was compared by using the individual curve method (ICM) versus the combined curve method (CCM). For this test, four stacks of known loads were placed along the feed trough at 1/5, 2/5, 3/5, and 4/5 of the length. Each stack consisted of four known loads (each weighing 375 to 400 g). One known load was removed at a time from each stack, and the remaining weight in the feeder was determined using the ICM or CCM curves. The weights obtained with ICM or CCM were compared to the actual weight, and the differences (measured vs. actual weights) were derived. Accuracy of the load cells for the water tanks and egg collectors was determined by using a standard known weight.

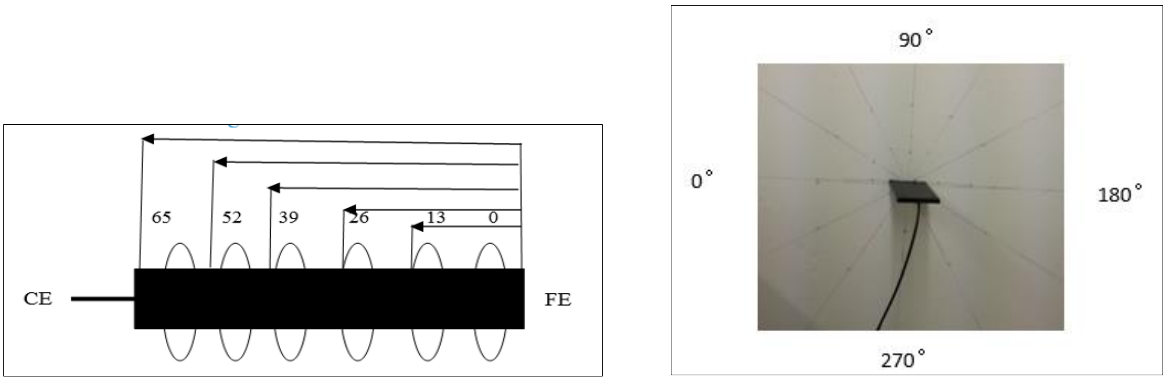
Detection Range of RFID tags

Although RFID antennas have a certain theoretical detection range (30 cm in this case for the feeder antennas), the range greatly depends on the position and orientation of the tags (Finkenzeller, 2003). To determine the detection range of the RFID system in our case, tests were performed that included possible positions and orientations of the tags. The maximum detection ranges for tags placed perpendicular or parallel to the antenna were determined at six evenly spaced locations in the lengthwise direction above the antenna at a power of 31.5 dBm.

In order to record all the hens when their heads are at the feed trough but avoid detecting signals from hens outside the feeder space (false reading), it is essential to set the power to a value that can read 100% of hens in the height range of approximately 10 cm above the antennas. A total of 66 tags attached to a plastic board at a height of 10 cm above the antennas (i.e., the distance between the bottom and top of the feed trough) and perpendicular to the antennas were used to determine the appropriate power value. Different power values from 31.5 to 0 dBm at 0.5 dBm increments were tested until a satisfying power value was found at which 100% of the tags could be read.

Uniformity of EM Signal across Antennas

To assess variability in detection range among the antennas, two SlimLine 8060 antennas, two IPJ-A0311-USA antennas, and two Square A1030 antennas were randomly selected and examined for detection ranges using the same set of tags. Signals at six points of the SlimLine 8060 antennas were selected from end to end, i.e., at 0, 13, 26, 39, 52, and 65 cm from free end (FE) to cable end (CE) (fig. 2.7a). Signals at three points of the IPJ-A0311-USA antennas were selected from end to end, i.e., at 0, 23, 46 cm from FE to CE. Similarly, signals at three points of the Square A1030 antennas were selected from end to end, i.e., at 0, 15, and 30 cm from FE to CE. Testing angles around the antennas were 0°, 30°, 60°, 90°, 120°, 150°, 180°, 210°, 240°, 270°, 300°, and 330° (fig. 2.7b). At each angle, the maximum distance at which a tag could be registered by the antenna was measured. Single-factor analysis of variance (ANOVA) was performed on each type of antenna using SPSS Statistics v20. This information also served as the baseline for adjusting the antennas power to minimize signal penetration into the colony area, which may lead to false registration of hens near the feeder but not feeding.



(a) **(b)**

Figure 2.7 *(a) Six signal points selected from the free end (FE) to the cable end (CE) and (b) 12 radial directions around and perpendicular to the antenna.*

Laying Hens and Validation of RFID System Performance

A total of 60 laying hens (Dekalb white, 66 to 70 weeks of age) were used for the system performance tests. To register the feeding and nesting behaviors of individual hens with the RFID system, each of the 60 hens in the colony wore a miniature tag with a unique ID (PT-103, tie-wrap tag passive Gen 2 UHF, TransTech Systems) on its neck (feeding) and another on its left leg (nesting) (fig. 2.8). During the test, lost tags were promptly replaced, and the day with lost tags was treated as missing data.

The hens were fed twice daily at 9:00 h and 17:00 h, with no restriction, with the feed provided by the farm that the hens came from. A photoperiod of 17 h light and 7 h dark

(17L:7D) was used, as practiced on the farm. Light intensity was 13 to 120 lux across the top tier colony. Eggs laid in the nest boxes rolled into the egg collectors and were manually removed once a day at 17:00 h. Manure was removed weekly. The hens were acclimatized in the colony for at least 5 d before behavioral testing started.

Figure 2.8 *RFID tags attached to hens.*

Validation of RFID Readings with Video Observation

The number of simultaneously feeding and nesting hens detected by the RFID system was compared to that determined by the video system. Hens were identified as feeding when they stood in front of the feeder and their heads faced the feed trough. Similarly, hens were identified as nesting when they were inside the nest box. Accuracy was calculated by the percentage of birds detected with the RFID system compared to the number of hens observed in video images for the same time point. Validations of the feeding and nesting behaviors were performed separately, with 38 and 78 episodes, respectively, during three consecutive days (5:00 h to 18:00 h each day). Specifically, feeding behavior was validated for every hour (with the exception of the first data point loss for the first day). For nesting behavior, because of the large number of birds accessing the nest boxes for oviposition at the same time, 30 min (rather than 60 min) episodes were used for the validation. The images from the two video cameras (each covering half of the top-tier colony) were extracted, undistorted, synchronized, and stitched together using the code developed in Matlab (fig. 2.9).

Figure 2.9 (a and b) *Original images recorded by two video cameras and (c) combined undistorted image.*

Feeding and Nesting Behaviors Monitored Using the RFID System

Time spent at the feeders and nest boxes as well as the maximum and average numbers of simultaneously feeding hens were examined over a 7-d period. With antenna power settings of 18 dBm for SlimLine 8060, 22 dBm for IPJ-A0311-USA, and 25 dBm for Square A1030, which deviated slightly from the power settings in the initial evaluation, the RFID system successfully registered hens when their feet were inside the nest area or when their heads were inside the feeder. However, a hen would not be registered at the feeder when she raised her head to swallow feed or momentarily withdrew from the feeder. Such brief intermittent breaks of up to 30 s were considered part of a feeding event. The 30 s threshold was determined from a histogram analysis of the feeding events from eight random tags that represented high, medium, and low levels of data collection. Time differences between two adjacent readings were determined for each of these tags, followed by generating and examining a histogram of the time differences. The same thresholding process was used to fill time gaps in nesting behaviors. In addition, manual labeling verification was made with eight randomly selected feeding or nesting hens.

Results and Discussion

Individual Load-Cell Scale Calibration

Comparison between the measured weights by ICM and CCM versus the actual weights loaded is shown in table 2.2. Both methods showed good accuracy, with less than 1.3% variation from the actual weight. The CCM had a better accuracy and less offset (0.1% ± 0.0%, 3.5 ± 1.4 g) and was therefore used to determine the feeder weight.

Sample Data of Feed and Water Use

Daily profiles of feed and water use for the group were obtained by calculating the changes in the weight of the feeders and water tank, and sample data are shown in figure 2.10. It can be seen that the hens continued to feed and drink throughout the light period, but no feeding or drinking activities occurred during the dark period. On this particular day, feed use by the 60 hens was 4.8 kg (an average of 80 g hen^{-1} d^{-1}), and water use was 9.1 kg (an average of 152 g hen^{-1} d^{-1}). Egg laying time and egg weight of the hens were also captured by the weighing system.

Table 2.2 *Weights derived by the individual curve or combined curve methods (ICM vs. CCM) for the load-cell scales versus actual values.*

	ICM			CCM		
Actual Weight	Derived Weight	Difference from Actual Weight		Derived Weight	Difference from Actual Weight	
(g)	(g)	(g)	(%)	(g)	(g)	(%)
6185.7	6205.9	20.2	0.3	6189.9	4.2	0.1
4635.7	4656.0	20.3	0.4	4640.3	4.6	0.1
3085.1	3105.2	20.1	0.7	3089.0	3.9	0.1
1542.6	1562.8	20.2	1.3	1544.0	1.4	0.1
Mean	-	20.2	0.7	-	3.5	0.1
±SD		±0.1	±0.4		±1.4	±0.0

Detection Range of RFID Tags

The maximal detection ranges were significantly affected by the tag position relative to the antenna (figs. 2.11 and 2.12). With the tags in the perpendicular position, they could be detected at up to 85 cm (SlimLine 8060) and 75 cm (IPJ-A0311-USA) above the antenna at the 31.5 dBm power setting. However, with the tags in the parallel position, the maximum height of tag detection was 8 cm (SlimLine 8060) and 6 cm (IPJ-A0311-USA). Because the position of the tag on a hen's neck may change throughout the day, an 8 cm detection range would not be adequate to ensure registration of hens at the feeder. Thus, the tag was attached to a collar and parallel to the hen's neck, which means that the tag would be perpendicular to the antenna when the hen's head entered the feed trough and during most feeding activities. In addition, the detection range changes along the antenna length, with the signal being weakest at the cable end. To better cover the weak signal points in the feed trough, the free end of the each antenna was positioned next to the cable end of the adjacent antenna.

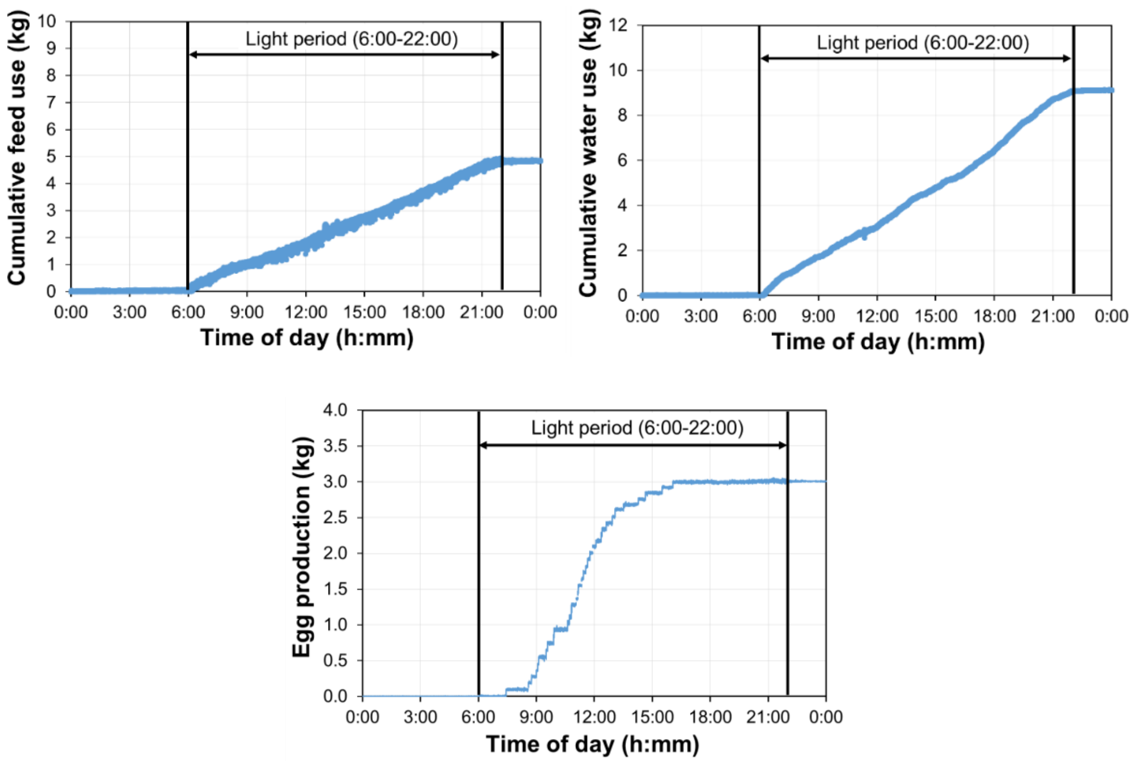


Figure 2.10 *Sample profiles of cumulative feed and water use and egg production for 60 hens in the enriched colony housing as measured by the load-cell scale systems.*

Figure 2.13 shows the percentage of tags registered by a IPJ-A0311-USA antenna with power settings of 20.0, 21.0, 22.0, 23.0, and 31.5 dBm at five heights above the antenna. All 66 tags were detected directly above the antennas in a feeder, while fewer than 10% were registered at 50 cm above the antennas. As the power decreased, so did the percentage of detected tags. Figure 2.13 shows that 22 dBm was the minimum power needed to achieve 100% detection of tags at 10 cm above the antenna.

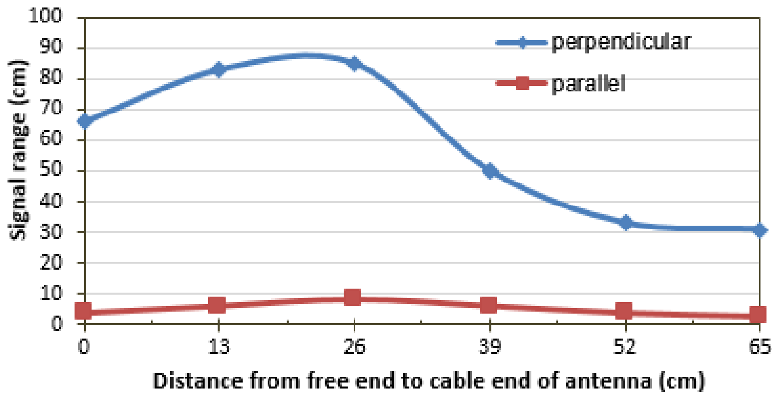


Figure 2.11 *Detection range distribution for 65 cm long SlimLine 8060 linear polarized antenna with two tag orientations (perpendicular to or parallel with the antenna) at 31.5 dBm power setting.*

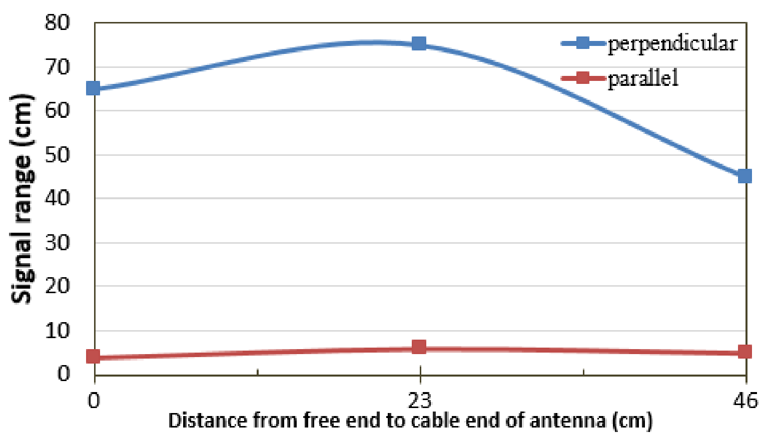


Figure 2.12 *Detection range distribution for 46 cm long IPJ-A0311-USA linear polarized antenna with two tag orientations (perpendicular to or parallel with the antenna) at 31.5 dBm power setting.*

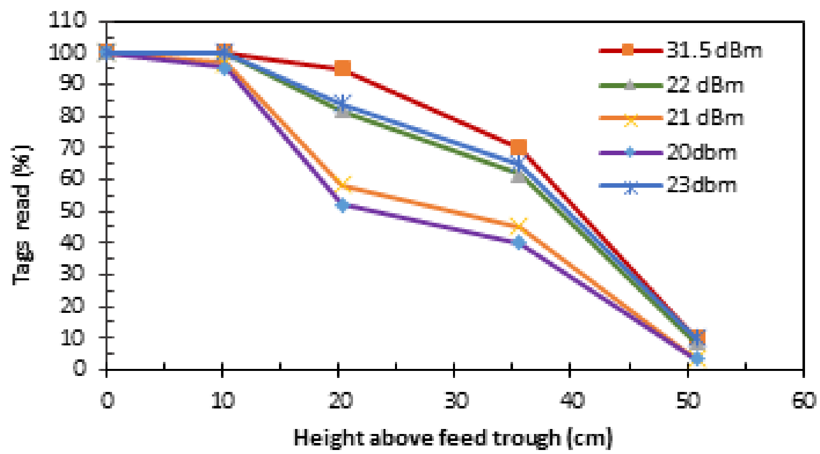


Figure 2.13 *Detection of the RFID tags by a IPJ-A0311-USA antenna at different power levels*

The SlimLine 8060 antenna had a wider detection range than the IPJ-A0311-USA antenna, as shown in tables 2.3 and 2.4. As a result, continual adjustment of the antenna power was made during the tests with hens. With power settings of 18 dBm (SlimLine 8060) and 22 dBm (IPJ-A0311-USA), the RFID system successfully registered hens when their heads were inside the feeders but not when their heads were raised to swallow feed nor when they momentarily withdrew while standing in front of the feeder.

Similarity among Antennas

The detection ranges around the SlimLine 8060, IPJ-A0311-USA, and Square A1030 antennas are shown in tables 2.3, 2.4, and 2.5, respectively. No significant differences were detected within the same type of antenna ($p > 0.05$). This operational characteristic made it possible to apply the same power to all the readers, thereby simplifying the system settings.

Table 2.3 *Tag detection range (cm) around two SlimLine 8060 antennas (A1 and A2) at 31.5 dBm power setting.*

Angle Around Antenna	0 cm		13 cm		26 cm		39 cm		52 cm		65 cm	
	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2	A1	A2
0°	36	36	49	49	49	49	41	41	31	31	18	18
30°	50	50	62	62	60	61	39	39	30	30	19	19
60°	62	62	70	70	79	78	50	50	30	30	19	19
90°	66	66	83	83	85	85	50	50	33	33	21	21
120°	62	62	74	74	80	80	50	50	31	31	19	19
150°	52	51	62	62	60	60	38	38	28	28	19	19
180°	41	41	52	52	52	52	44	45	29	29	17	17
210°	31	31	41	40	53	53	29	28	21	21	15	16
240°	38	39	49	49	52	52	26	26	21	21	18	18
270°	32	32	42	42	42	42	30	31	25	25	21	21
300°	30	30	40	40	39	39	28	28	20	21	18	18
330°	32	32	45	45	43	43	27	27	21	21	15	15

Distance between Free End and Cable End of Antenna spans the 0 cm to 65 cm columns.

Table 2.4 *Tag detection range (cm) around two IPJ-A0311-USA antennas (B1 and B2) at 31.5 dBm power setting.*

Angle Around Antenna	Distance between Free End and Cable End of Antenna					
	0 cm		23 cm		46 cm	
	B1	B2	B1	B2	B1	B2
0°	33	33	39	42	33	32
30°	48	47	52	55	29	28
60°	60	62	69	70	40	42
90°	63	65	72	75	40	45
120°	58	60	70	72	40	40
150°	50	54	50	53	28	29
180°	42	44	42	45	25	34
210°	31	29	33	35	19	18
240°	39	38	43	45	17	16
270°	32	30	35	35	21	20
300°	30	28	32	33	18	18
330°	35	33	35	35	17	17

Table 2.5 *Tag detection range (cm) around two Square A1030 antennas (C1 and C2) at 31.5 dBm power setting.*

Angle Around Antenna	Distance between Free End and Cable End of Antenna					
	0 cm		15 cm		30 cm	
	C1	C2	C1	C2	C1	C2
0°	0	0	0	0	0	0
30°	10	9	18	18	22	23
60°	6	6	15	15	20	20
90°	5	5	6	6	18	18
120°	9	8	18	17	16	16
150°	13	13	22	21	15	16
180°	0	0	0	0	0	0
210°	7	6	0	0	0	0
240°	5	5	0	0	0	0
270°	6	6	0	0	0	0
300°	6	6	0	0	0	0
330°	8	8	0	0	0	0

Validation of RFID Readings with Video Observation

A threshold time of 30 s for inclusion of RFID data in a single behavioral event (feeding or nesting) was identified to obtain 95% coverage of the data collected by the RFID system, as shown in figure 2.14. This 30 s threshold was verified visually for feeding and nesting behaviors. Visual observations revealed that it took the hens 68.1 ±12.5 s (mean ± SD, n = 8) to leave the feeder, perform other activities (e.g., scratching, perching, drinking water, nesting), and return to the feeder. Similarly, it took the hens 95.1 ± 23.5 s (mean ±SD, *n* = 6) to leave and return to the nest box (two of the eight randomly selected birds did not return to the nest box during the 5 min visual verification period). The 30 s threshold was used to fill the time gaps in the RFID readings when characterizing feeding and nesting behaviors (e.g., feeding or nesting duration, and frequency of feeder and nest box visit).

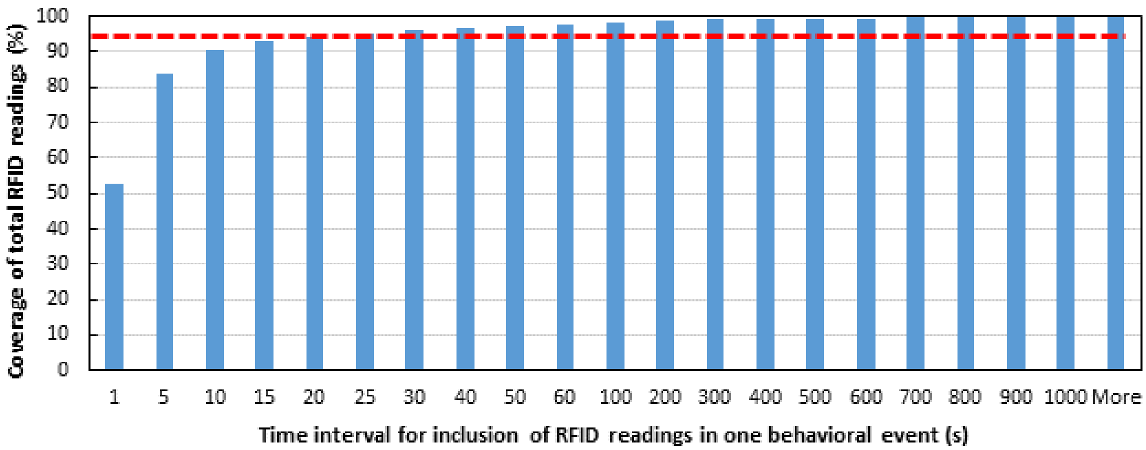


Figure 2.14 *Coverage of RFID readings versus time interval for inclusion in one behavioral event. The dashed line indicates 95% coverage of the RFID readings.*

The results obtained with the RFID system were compared against video observation for both feeding and nesting behaviors. The overall accuracy of the RFID system relative to the video observation was (mean ± SD) 92.1% ± 6.4% (*n* = 38) for feeding behavior and 91.4% ± 1.7% (*n* = 78) for nesting behavior, demonstrating reasonable effectiveness of the RFID system. This performance was comparable to the result of Thurner et al. (2008), who reported an average identification rate of 89.8% using HF transponders to register laying behavior of individual hens. Sales et al. (2015), using a passive LF RFID system, reported detection rates (mean ±SD) of 91.0% ± 2.6% for trials with groups of hens and 85.8% ± 8.0% for trials with individual hens when measuring total compartment (1.2 m × 1.2 m × 1.2 m) occupancy. Reiners et al. (2009) reported an identification rate of 97.3% using an HF RFID system for identification of individual weaned piglets (20 animals in a group) at the feed trough.

In addition to the direct comparison, a regression analysis was performed to relate the results from the two measurement systems. Validation of the RFID system for the feeding behavior assessment showed that the correlation coefficient between the RFID and camera systems was 0.984 (slope of 1.008 ± 0.017), reflecting good agreement between the two measurement methods (fig. 2.15). A high correlation coefficient of 0.989 (slope of 0.950 ± 0.014) between the two methods was also found for the nesting behavior analysis (fig. 2.15).

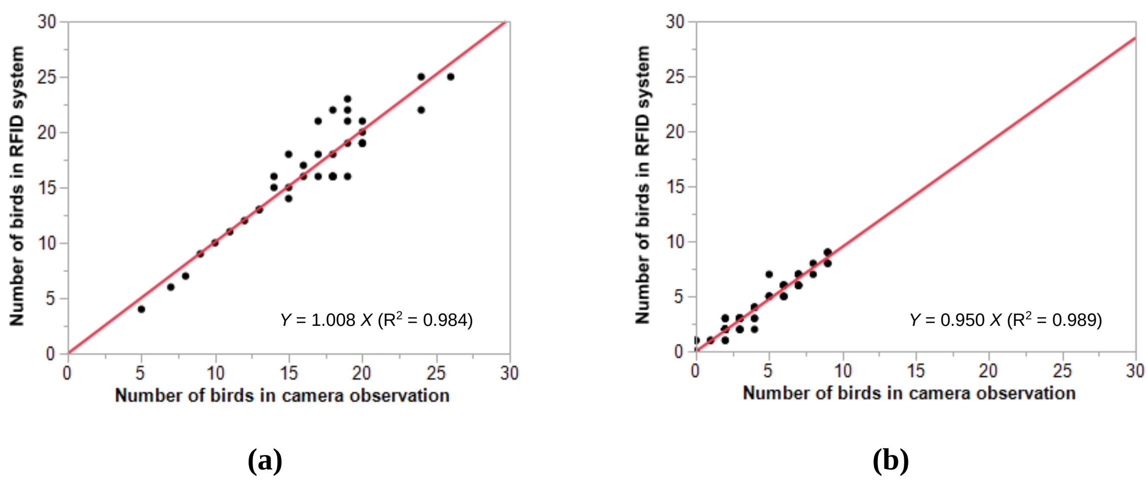


Figure 2.15 *Relationship of values obtained with the RFID system versus video observation for (a) the number of feeding hens (feeding behavior) and (b) the number of hen using the nest (nesting behavior).*

Sample Data of Feeding and Nesting Behaviors Monitored with the RFID System

Figure 2.16 shows the average time spent at the feeder by the 60 hens over the course of seven experimental days. A summary of the feeding behavior data (time spent plus number of hens feeding simultaneously) is listed in table 2.6. The daily time spent at the feeder by the hens (mean ± SD) was 310 ± 92 min hen^{-1} d^{-1}. Cook et al. (2005) found that hens housed at different stocking densities (348, 387, 426, and 465 cm^2 hen^{-1}) in conventional cages had a daily feeding time of 180 to 240 min. Persyn et al. (2004) reported that individually housed 77-week-old hens showed a mean daily feeding time of 198 ± 24 min. Compared with the previous conventional cage tests, the hens in the current ECH and with larger feeder space showed longer feeding time. Huon et al. (1986) qualitatively reported that increasing feed trough space (presumably improving welfare) resulted in longer feeding-bout duration and feeding time.

The maximum number (mean ± SD) of hens feeding simultaneously was found to be 36 ± 3, corresponding to 61% of the total hen population in the colony (table 2.6). Appleby (2004) called for feeder space of at least 12 cm hen^{-1} and allowing all hens to feed simultaneously. Based on the results of this preliminary study, all hens never ate at the same time. Further research is therefore warranted to quantify the feeder space needed by group-housed hens, especially in alternative hen housing systems that feature enrichments (perches, scratch area, etc.).

As indicated by the data in figure 2.17, the hens displayed a daily nesting time of 56 ± 45 min. This result parallels the report by Stämpfli et al. (2011), who found that birds can spend 10 to 90 min when laying an egg.

Assessment of the impact of feeder space on feed and water use and egg production with the system is ongoing and will be presented in future articles. Nesting behaviors obtained with this monitoring system, such as time spent in the nest boxes and oviposition time and place, were presented in a separate publication (Oliveira et al., 2016).

Table 2.6 *Feeding behaviors of hens in the enriched colony housing.*

Day	Total No. of Hens	Time Spent at Feeder (min hen^{-1} d^{-1})	No. of Hens Feeding Simultaneously (% total) Maximum	Average
1	60	326	42 (70%)	19 (32.2%)
2	60	313	34 (57%)	19 (31.0%)
3	60	315	37 (62%)	19 (31.2%)
4	60	303	37 (62%)	18 (30.0%)
5	59	300	33 (56%)	18 (29.8%)
6	59	301	34 (58%)	18 (29.8%)
7	59	311	35 (59%)	18 (30.8%)
Mean	60	310	36 (61%)	18 (30.7%)
SD	0.5	9	3 (4.4%)	1 (0.8%)

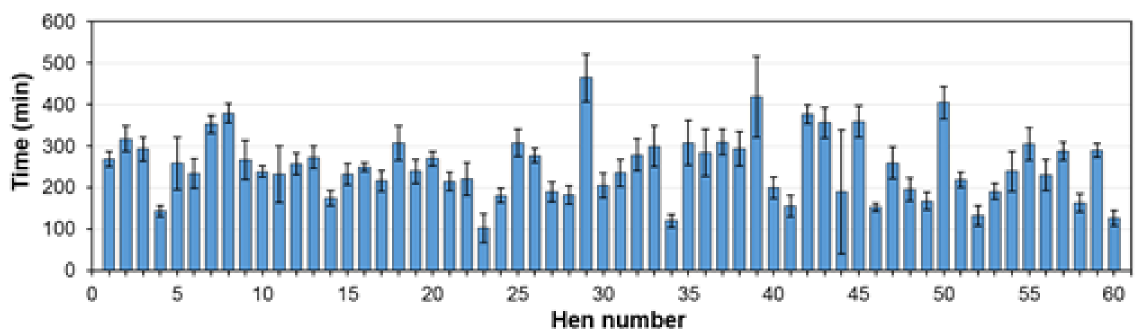


Figure 2.16 *Daily time spent at the feeder by each of 60 hens in enriched colony housing over a 7 d period (vertical bars are standard deviations)*

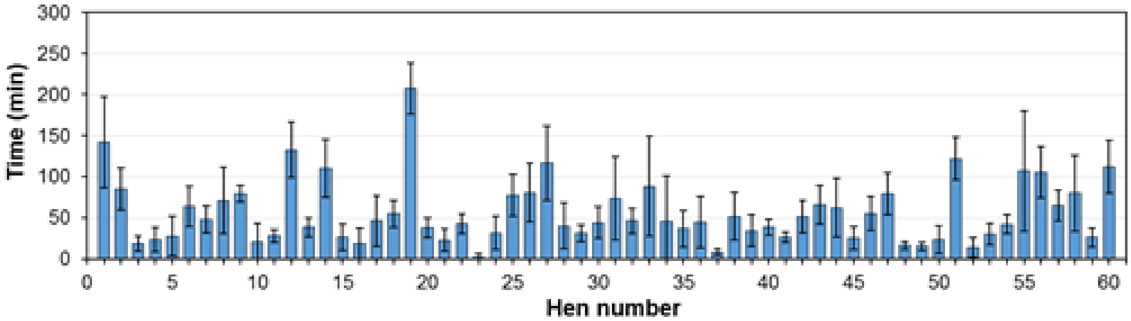


Figure 2.17 *Daily average time spent in the nest box by each of 60 hens in enriched colony housing over a 5 d period (vertical bars are standard deviations)*

Conclusions

A UHF RFID system for characterizing feeding and nesting behaviors of individual hens in an enriched colony setting has been developed and tested. The performance of the RFID system was validated by a video system. The results demonstrated that the system can be used to characterize dynamic poultry feeding and nesting behaviors. The system allows for assessing the impact of housing and management factors, such as feeder space and stocking density, on feeding behaviors and feed intake of laying hens, and the number of hens feeding at the same time. The resulting information will contribute to the development or improvement of guidelines for housing system design and management to ensure animal welfare and efficient use of resources.

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References

Appleby, M. C. (2004). What causes crowding? Effects of space, facilities, and group size on behaviour, with particular reference to furnished cages for hens. Animal Welfare, 13(3), 313-320.

Ben Sassi, N., Averos, X., & Estevez, I. (2016). Technology and poultry welfare. Animals, 6(10), 62. https://doi.org/10.3390/ani6100062

Bracke, M. B. M., & Hopster, H. (2006). Assessing the importance of natural behavior for animal welfare. J. Agric. Environ. Ethics, 19(1), 77-89. https://doi.org/10.1007/s10806-005-4493-7

Brown-Brandl, T. M., & Eigenberg, R. A. (2011). Development of a livestock feeding behavior monitoring system. Trans. ASABE, 54(5), 1913-1920. https://doi.org/10.13031/2013.39832

Cappai, M. G., Picciau, M., Nieddu, G., Bitti, M. P., & Pinna, W. (2014). Long-term performance of RFID technology in the large-scale identification of small ruminants through electronic ceramic boluses: Implications for animal welfare and regulation compliance. Small Ruminant Res., 117(2), 169-175. https://doi.org/10.1016/j.smallrumres.2013.12.031

Catarinucci, L., Colella, R., Mainetti, L., Patrono, L., Pieretti, S., Secco, A., & Sergi, I. (2014). An animal tracking system for behavior analysis using radio frequency identification. Lab Animal, 43(9), 321-327. https://doi.org/10.1038/laban.547

Cook, R. N., Xin, H., & Nettleton, D. (2006). Effects of cage stocking density on feeding behaviors of group-housed laying hens. Trans. ASABE, 49(1), 187-192. https://doi.org/10.13031/2013.20237

Finkenzeller, K. (2003). RFID handbook: Fundamentals and applications in contactless smart cards, radio frequency identification, and near-field communication (3rd ed.). West Sussex, UK: John Wiley & Sons. https://doi.org/10.1002/0470868023

Harmon, J. D. (2008). Rigid plastic tubes for pit ventilation. Ames, IA: Iowa State University. Retrieved from https://store.extension.iastate.edu/Product/Rigid-Plastic-Tubes-for-Pit-Ventilation

Huon, F., Meunier-Salaün, M.-C., & Faure, J.-M. (1986). Feeder design and available feeding space influence the feeding behaviour of hens. Appl. Animal Behav. Sci., 15(1), 65-70. https://doi.org/10.1016/0168-1591(86)90023-7

Maselyne, J., Saeys, W., De Ketelaere, B., Mertens, K., Vangeyte, J., Hessel, E. F., ... Van Nuffel, A. (2014a). Validation of a high-frequency radio frequency identification (HF RFID) system for registering feeding patterns of growing-finishing pigs. Comput. Electron. Agric., 102, 10-18. http://dx.doi.org/10.1016/j.compag.2013.12.015

Maselyne, J., Van Nuffel, A., De Ketelaere, B., Vangeyte, J., Hessel, E. F., Sonck, B., & Saeys, W. (2014b). Range measurements of a high-frequency radio frequency identification (HF RFID) system for registering feeding patterns of growing-finishing pigs. Comput. Electron. Agric., 108, 209-220. http://dx.doi.org/10.1016/j.compag.2014.08.006

Mench, J. A., Sumner, D. A., & Rosen-Molina, J. T. (2011). Sustainability of egg production in the United States: The policy and market context. Poultry Sci., 90(1), 229-240. https://doi.org/10.3382/ps.2010-00844

Nakarmi, A. D., Tang, L., & Xin, H. (2014). Automated tracking and behavior quantification of laying hens using 3D computer vision and radio frequency identification technologies. Trans. ASABE, 57(5), 1455-1472. https://doi.org/10.13031/trans.57.10505

Oliveira, J., Xin, H., Zhao, Y., Li, L., Liu, K., & Glaess, K. (2016). Nesting behaviors and egg production pattern of laying hens in enriched colony housing. ASABE Paper No. 162456546. St. Joseph, MI: ASABE. https://doi.org/10.13031/aim.20162456546

Persyn, K. E., Xin, H., Nettleton, D., Ikeguchi, A., & Gates, R. S. (2004). Feeding behaviors of laying hens with or without beak trimming. Trans. ASAE, 47(2), 591-596. https://doi.org/10.13031/2013.16040

Reiners, K., Hegger, A., Hessel, E. F., Böck, S., Wendl, G., & Van den Weghe, H. F. A. (2009). Application of RFID technology using passive HF transponders for the individual identification of weaned piglets at the feed trough. Comput. Electron. Agric., 68(2), 178-184. http://dx.doi.org/10.1016/j.compag.2009.05.010

Sales, G. T., Green, A. R., Gates, R. S., Brown-Brandl, T. M., & Eigenberg, R. A. (2015). Quantifying detection performance of a passive low-frequency RFID system in an environmental preference chamber for laying hens. Comput. Electron. Agric., 114, 261-268. http://dx.doi.org/10.1016/j.compag.2015.03.008

Samad, A., Murdeshwar, P., & Hameed, Z. (2010). High-credibility RFID-based animal data recording system suitable for small-holding rural dairy farmers. Comput. Electron. Agric., 73(2), 213-218. http://dx.doi.org/10.1016/j.compag.2010.05.001

Stämpfli, K., Roth, B. A., Buchwalder, T., & Fröhlich, E. K. (2011). Influence of nest-floor slope on the nest choice of laying hens. Appl. Animal Behav. Sci., 135(4), 286-292. https://doi.org/10.1016/j.applanim.2011.10.008

Thurner, S., Wendl, G., Bock, S., Frohlich, G., & Preisinger, R. (2008). Simultaneous registration of hens in group nest boxes with a HF transponder system to evaluate the laying behaviour. Presented at the Intl. Conf. on Agricultural Engineering (AgEng 2008).

Kyoto, Japan: International Commission on Agricultural and Biosystems Engineering (CIGR).

Tu, X., Du, S., Tang, L., Xin, H., & Wood, B. (2011). A real-time automated system for monitoring individual feed intake and body weight of group-housed turkeys. Comput. Electron. Agric., 75(2), 313-320. http://dx.doi.org/10.1016/j.compag.2010.12.007

UEP. (2016). Animal husbandry guidelines for U.S. egg laying flocks. Johns Creek, GA: United Egg Producers Certified. Retrieved from www.uepcertified.com.

Voulodimos, A. S., Patrikakis, C. Z., Sideridis, A. B., Ntafis, V. A., & Xylouri, E. M. (2010). A complete farm management system based on animal identification using RFID technology. Comput. Electron. Agric., 70(2), 380-388. http://dx.doi.org/10.1016/j.compag.2009.07.009

Zhao, Y., Shepherd, T. A., Swanson, J. C., Mench, J. A., Karcher, D. M., & Xin, H. (2015). Comparative evaluation of three egg production systems: Housing characteristics and management practices. Poultry Sci., 94(3), 475-484. https://doi.org/10.3382/ps/peu077

CHAPTER 3. IMPACT OF FEEDER SPACE ON LAYING HEN FEEDING BEHAVIOR AND PRODUCTION PERFORMANCE IN ENRICHED COLONY HOUSING

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Abstract

Current feeder space recommendations in laying hen welfare guidelines are inconsistent among and within countries. One determining criterion forming the recommendations (e.g., 12.0 cm/hen for the EU guideline) is that all birds can feed simultaneously. However, if there are other resources in the environment, as in enriched colony housing (ECH), it is unknown whether group-housed hens will choose to feed simultaneously. This study assesses the impact of feeder space on feeding behavior of 60 laying hens (W-36) in ECH using a UHF RFID-based tracking system. The feeder spaces investigated were 12.0, 9.5, 8.5, and 6.5 cm/hen, achieved by blocking portions of the overall feeder access to keep hens at the same stocking density. Each feeder space treatment, randomly assigned over the course of the experiment, lasted for seven consecutive days. Feeding behaviors were characterized as daily time spent at the feeder (TS, min/hen-d), daily frequency of visits to the feeder (FV, #/hen-d), and maximum or average percentage of hens feeding simultaneously (MPB, APB, %). Group-average daily feed intake (FI, g/hen-d), water use (WU, g/hen-d), and hen-day egg production (HDEP, %) were also measured. The results revealed that at 12.0 cm/hen, where unoccupied feeder space was present, a maximum of $59.0 \pm 1.4\%$ (average of

$31.7 \pm 0.3\%$) hens fed simultaneously. No significant differences were detected among 12.0, 9.5, and 8.5 cm/hen in TS (293 ± 10, 286 ± 10, and 281 ± 10 min/hen-d) and MPB (59.0 ± 1.4, 57.3 ± 1.4, and $53.3 \pm 1.4\%$) ($P > 0.05$). The outcome of no significant differences also held true between 12.0 and 9.5 cm/hen in APB (31.7 ± 0.3 *vs.* $30.8 \pm 0.3\%$) and between 9.5 and 8.5 cm/hen in all response variables measured ($P > 0.05$). However, there were significant differences in APB between 6.5 cm/hen and all other treatments; in TS and FV between 6.5 and 9.5 cm/hen; and in MPB between 6.5 and 12 cm/hen ($P < 0.05$). Considerable inter-hen variability was observed in TS (CV varying from 28.0 to 32.1%) and FV (CV varying from 26.5 to 27.8%). All the feeder spaces tested showed no significant impact on FI, WU, or HDEP ($P > 0.05$). The results revealed that synchronous feeding of hens in the ECH did not increase with increasing feeder space. However, it is worth noting that lower feeder space may lead to aggression or frustration which was not quantified in the current study.

Keywords: Alternative housing, hen welfare guideline, space allowance, synchronous feeding, radio-frequency identification

Implications

Provision of proper feeder space is a key parameter in hen housing design. However, feeder space guidelines from hen welfare standpoint vary considerably among and within countries. Results of this study show that not all hens in the enriched colony housing choose to feed simultaneously and that synchronous feeding of the hens did not increase with increasing feeder space. These findings help guide housing design for increased efficiency of resource utilization while maintaining production performance and accommodating certain feeding behaviors of the hens. It should be noted that the study did not cover the full range of welfare measurements such as aggression or frustration that may arise from reduced feeder space.

Introduction

Feeder space is an important parameter in the design and management of poultry production facilities. Insufficient feeder space can cause competition or aggression among animals, which will adversely affect their well-being (Sirovnik *et al.*, 2018). Excessive feeder space leads to inefficient use of resources and may also lead to unnecessary complication in the system design and management. Global feeder space recommendations centered on time and synchrony at the feeder for laying hens have been based on scarce scientific data. Appleby (2004) reported that with the lack of scientific information, feeder space requirement should assume that all birds have space enough to feed simultaneously, as it is for perch design: 14 cm per bird for medium and 12 cm per bird for light hybrids.

Cook *et al*. (2006) studied feeding behavior and production performance of laying hens in conventional cages and found no effect of cage stocking density (and consequently feeder space) on feed intake, feeding time per hen, number of meals ingested per day-cage, meal size (amount of feed intake per meal), meal duration, ingestion rate, and number of hens feeding per meal. Thogerson *et al.* (2009a and 2009b) evaluated the effects of feeder space allocation (achieved from different stocking densities) on productivity, physiology, feeding behavior, aggression, feather score, body weight (BW), and mortality of W-36 laying hens in conventional cages. Hens with reduced feeder space were found to spend less time feeding and show less synchronization at the feeder and have higher feed intake (probably due to wastage), but no significant effects were observed on aggression, mortality, BW, BW uniformity, bone mineralization, or physiologic stress.

Mench and Blatchford (2014) determined the overall space usage by laying hens using kinematic analysis, and reinforced that the space requirements for group-housed hens must account for the tendency of hens in a flock to synchronize their behavior. Widowski *et al*. (2016) reported that further research is necessary to determine the space requirements of hens for particular resources (e.g., feeders, litter or scratch areas, and nests), rather than space allowance *per se*, in these more complex housing systems. According to the study by Widowski *et al*. (2017a), the synchrony of laying hens at the feeder in furnished cages was not affected by cage size or space allowance. On the other hand, a recent study by Sirovnik *et al.* (2018) reported that in aviary systems, the increase in feeder space significantly increased the synchrony at the feeder and feeding bouts.

Because of the scarce research information regarding hen feeder space requirement, especially in emerging alternative hen housing systems, different organizations or certifying entities may have different sets of guidelines to meet the local or specific animal welfare standards. Table 3.1 lists the comparison of feeder space guidelines adopted in the United States, Canada, and the European Union (EU). To address these discrepancies, additional research is warranted, as alternative hen housing systems are increasingly adopted worldwide.

The primary objective of this research was to evaluate the impact of feeder space on feeding behaviors of laying hens in an enriched colony housing (ECH), focusing on 1) daily time spent (**TS**, min/hen-d) at the feeder, 2) daily frequency of feeder visits (**FV**, #/hen-d), and 3) synchronization of feeding as measured by maximum and average percentage of hens feeding simultaneously (**MPB** and **APB**, %). The secondary objective was to quantify the variability in feeding behaviors (TS and FV) among the individual hens. The tertiary objective was to assess the effect of feeder space on production performance as measured in group-average daily feed intake (**FI**, g/hen-d), water use (**WU**, g/hen-d), and hen-day egg production (**HDEP**, %). Our hypotheses were that a) feeding behavior (TS, FV, MPB, and APB) and production performance (FI, WU, and HDEP) of the hens would not be affected by the feeder spaces tested; and b) not all hens would feed simultaneously.

Table 3.1 *Feeder space guidelines for laying hens adopted in USA, Canada, and EU*

Guideline	Minimum feeder space recommendation
United Egg Producers (UEP) – USA	7.6 cm per hen (1 side) or 3.8 cm (2 sides) – Cage free Enough space so all birds could feed simultaneously – enriched colony housing (ECH) and conventional cages (United Egg Producers (UEP), 2017)
American Humane Certified – USA	7.6 cm per hen (1 side) or 3.8 cm (2 sides) – Cage free 9.4 cm per hen (1 side) or 4.7 cm per hen (2 sides) – ECH (American Humane Certified, 2017a and 2017b)
Humane Farm Animal Care (HFAC) – USA	10 cm per hen (1 side) or 5 cm (2 sides) – Cage free (Humane Farm Animal Care (HFAC), 2017)
National Farm Animal Care Council (NFACC) – Canada	7.0 cm per hen – Cage free and ECH (National Farm Animal Care Council (NFACC), 2017)
EU laying hen directive 1999/74/EC – European Union	10 cm per hen – Cage free 12 cm per hen – ECH (European Commission, 1999)

Materials and Methods

Before commencement of the study, the experimental protocol had been approved by the Iowa State University Institutional Animal Care and Use Committee – IACUC (Log # 6-15-8038-G). This application and approval process complies with standard federal policies for conducting research or teaching experiments involving use of live animals.

Animals and housing

Sixty W-36 (white) laying hens at 21 weeks of age (WOA) were obtained from an enriched colony hen house at a layer farm in central Iowa and transported to our research laboratory at Iowa State University. The pullets (young hens before lay) had been reared in standard rearing cages: 0.61 m long × 0.76 m wide, 18 birds/cage (258 cm^2/bird), 2 nipple drinkers, feed trough with available space of 3.4 cm/bird, and galvanized wire-mesh floor. At 17 WOA, the pullets were transferred to the commercial enriched colony hen houses equipped with Big Dutchman enriched colony system (AVEC II, Big Dutchman, Vechta, Germany), where they were housed in 60-bird colonies at an average stocking density of 750 cm^2/bird.

One double-tier ECH module (Big Dutchman), measuring 3.73 m long × 1.91 m wide × 1.91 m high (Figure 3.1), was used in this experiment. The colony in each tier was equipped with perches, nest boxes, and scratch pads. Feed troughs were on both sides of the colony. Manure was collected on a plastic tarp placed underneath the colony tier and removed once or twice a week. The nominal capacity of the colony was 60 hens, with resource allowance of 750 cm^2 of floor area/hen, 85.4 cm^2 of nest area/hen, 49.0 cm^2 of scratch pad area/hen, 10 hens per nipple drinker, and 15.7 cm of perch per hen. The research lab used an automatically controlled ventilation system that consisted of an environment controller (Varifan ECS-3C, Quebec, Canada), two variable-speed exhaust fans (Multifan, Vostermans Ventilation, Bloomington, IL), and a supplemental heating and cooling system. The room temperature was maintained at 23 (±1) ºC throughout the experiment.

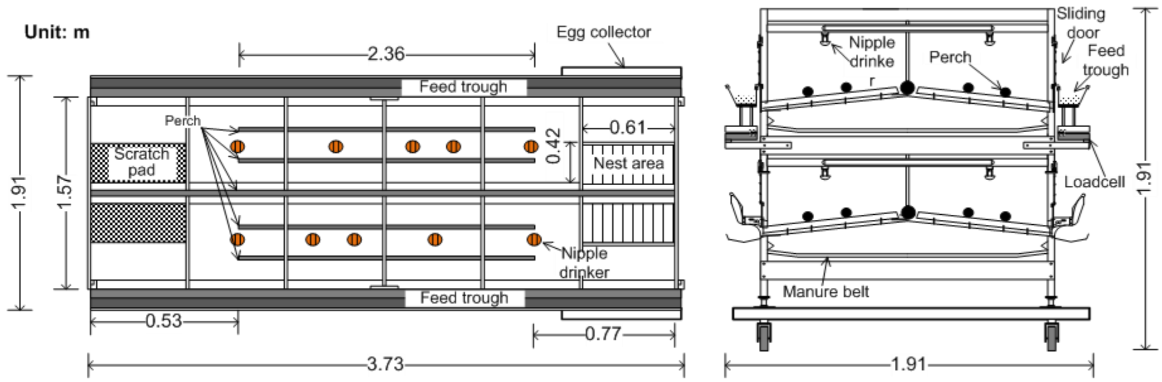

Figure 3.1 *Top-view (left) and side-view (right) schematic drawings of the enriched colony housing (ECH) module used for the laying hens (W-36) in the study.*

The hens were fed twice daily at 09:00h and 17:00h (*ad libitum*) with the same feed as used on the commercial farm where the hens came. For each feeding time approximately 10 kg of feed was added manually. The hens were able to move their heads horizontally along the feed troughs. A photoperiod of 16 hr light and 8 hr dark (16L:8D) as practiced on the farm was used, with lights-on at 05:00h and lights-off at 21:00h. A 15-minute dimming period was used as practiced on the farm. Light intensity was 13-120 lux across the top tier colony, with the lower intensity near the nest boxes area and the higher intensity near the scratch pads area. All eggs laid in the nest boxes rolled into two egg collectors (one on each colony side), and they were manually removed once a day at 17:00h.

Experimental design

The “as-is” feeder space of the experimental ECH was 12.0 cm/hen, reflecting the EU guideline, designated as the control in this study. Three additional levels of feeder space were tested, i.e., 9.5, 8.5, and 6.5 cm/hen. The reduced feeder spaces (9.5, 8.5, and 6.5 cm/hen) were achieved by partially blocking the hen's access to the feeder with metal wire mesh pieces placed intermittently along the feeder, i.e., one mesh installed per window of the ECH. All other resources (nest area, scratch pad area, and perches) and hen stocking density remained the same for all four feeder spaces.

The four feeder spaces were tested one at a time, with the treatment sequence randomized (12, 6.5, 8.5 and 9.5 cm/hen). Testing for each feeder space lasted 7 days, with the first 2 days as the acclimation period and data from the last 5 days used in evaluating the treatment effect. Prior to application of the feeder space treatments, the hens had been acclimatized to the ECH environment for 14 days. Acclimation of the hens to the ECH environment or a new FS treatment was verified by monitoring daily feed and water intake and egg production. Individual hens were considered as the experimental units for the feeding behavior response variables of TS and FV, whereas the ECH was the experimental unit for the response variables of MPB, APB, FI, WU, and HDEP.

Instrumentation

The top tier colony (where the hens were housed) was instrumented to monitor real-time feed and water use, record egg production (timing and number), and track the individual hens. Load-cell scales (Rice Lake Weighing Systems, Rice Lake, WI) were used to automatically and continuously (every second) weigh the feeders, water tank and egg collectors. Weight of the drinking-water tank was automatically monitored with one calibrated suspension load-cell scale; whereas each feeder trough was supported and weighed with three calibrated compression load-cell scales. The water tank weight had a maximum measurement error of 7.3 g (< 0.1% of daily WU), whereas weight of the feeder troughs had a maximum measurement error of 4.2 g (< 0.1% of daily FI). Details on calibration of the load-cell scales were described by Li *et al*. (2017).

Two cameras (IP Pro 3 Megapixel Bullet, DSS-BFR3MP, Backstreet Surveillance, Salt Lake City, UT) were installed on the ceiling above the ECH module and used to record the hen behaviors at 2 frames per second (fps). Video files were stored in 8 terabyte storage (two hard drives) of an NVR system (DSS-NVR5816, Backstreet Surveillance, Salt Lake City, UT). Air temperature, relative humidity (RH) and carbon dioxide (CO_2) of the hen room were measured and recorded. All sensors were connected to a compact Field Point Module, and data recorded using a LabVIEW program (National Instruments Co., Austin, TX).

Radio-frequency identification (RFID) system

RFID technology, using electromagnetic fields to identify tags attached to objects, has been applied to evaluate the foraging behavior of individual laying hens (Sales *et al*., 2015); to monitor individual feed intake of group-housed turkeys (Tu *et al.*, 2011); to assess pre-laying behavior of domestic hens (Ringgenberg *et al*., 2015); and to evaluate the range utilization of laying hens in free-range systems (Hartcher *et al*., 2016; Campbell *et al*., 2017). RFID technology has also been used in combination with 3D vision camera to quantify behavior of individual laying hens in group housing (Nakarmi *et al*., 2014). RFID systems can be used to assess individual behavior of laying hens for a longer period than with standard video assessment method, and thus provide more precise information on behavioral responses such as daily time spent at feeder, frequency of visits and synchrony at feeder throughout the day.

In this study, an ultra-high frequency (UHF) RFID system (TransTech Systems, Aurora, OR) consisting of four elements – antennas, tags, readers, and a data acquisition (DAQ) system was used. The antennas were placed at the bottom of feed troughs and covered with plastic boards. Each feed trough had six antennas (four SlimLine 8060 – 864-869 MHz/902-928 MHz, 65 cm long × 8.6 cm wide × 0.8 cm thick each; and two IPJ-A0311-USA, 46 cm long × 8.9 cm wide × 1.9 cm thick each, TransTech Systems, Aurora, OR) aligned in series covering the length of the feeder. The antennas were situated such that the tag (902-928 MHz, PT-103, tie-wrap tag passive Gen 2 UHF, TransTech Systems) attached to the hen's neck could be registered when the hen was present at the feeder.

All antennas were connected to four 4-channel readers (ThingMagic Mercury M6, 865-928 MHz operating frequency, TransTech Systems). Two of the four readers received output from the six antennas for one (west) feeder while the other two readers received output from the six antennas for the other (east) feeder. The readers were connected to a host computer that processed the tag data through an RJ45 (Registered Jack, 10/100 Base-T Ethernet). The tag protocol was EPCglobal Gen 2 (ISO 18000-6C) with Digital Rights Management (DRM). The data acquisition program was written in C# (C Sharp) based on Application Programming Interface (API) and the data were stored as text files.

Each hen was fitted with a rubber-band collar. The collars were placed on the hens two days after they had been in the experimental ECH. Initially some of the collars needed to be replaced or adjusted daily. Hens often had their beaks stuck on the collars while trying to take them off, which might require human intervention to free the stuck beak. It took about 7 days for the hens to get used to the collars, after which the RFID tags were attached to the collars. This approach avoided the occasional loss of the tags in the manure during the acclimation period.

The hens were considered feeding when their bodies were in front of the feeder and their heads were facing the feed trough. The RFID system successfully registered hens when their heads were inside the feeder but not when they raised to swallow feed or momentarily withdrew while occupying the space in front of the feeder. These intermittent brief breaks up to 30 s were considered as part of a feeding event. Details on the RFID configuration, data analysis, system performance and validation were described by Li *et al*. (2017).

Measurements and data processing

Load-cell scales were used to determine the group-level FI and WU. The data were collected every second via a program developed in LabVIEW, and then processed using EXCEL VBA programs. The uncertainty associated with these variables was $< 0.1\%$ of the total weight measured. HDEP was calculated as the ratio of total number of eggs laid per day to the corresponding number of hens in the colony. Data collected from the RFID system consisted of time (hh:mm:ss:ms) and place (antenna #) when a specific bird (tag number) was detected feeding.

Each reader generated one txt file, and a total of four reader files were collected to cover both feeders. The number of hens detected by the RFID system was compared with and validated by human visual labeling through the video system. The overall accuracy of the RFID system relative to the video observation was (mean $\pm$ SD) $92.1 \pm 6.4\%$ ($n = 38$) (Li *et al*., 2017).

Statistical analysis

The data for APB, MPB, FI, WU, and HDEP were analyzed using SAS 9.4 and one-way analysis of variance (ANOVA). The ANOVA was shown to be adequate for the data by the plot of residuals versus predicted values and the normal probability plot of the residuals. The data for TS and FV were analyzed as repeated measures using SAS 9.4 and the following mixed linear model:

$$y_{ijk} = \mu + \alpha_i + b_j + \gamma_k + (\alpha\gamma)_{ik} + e_{ijk},$$

where y_{ijk} is the response (TS or FV) value on day k for bird j assigned to treatment i; μ is the overall mean effect; α_i is the fixed effect of treatment i; b_j is the random effect of bird j; γ_k is the fixed effect of experimental day k; $(\alpha\gamma)_{ik}$ is the fixed interaction effect between treatment i and day k; and e_{ijk} is the random error associated with bird j assigned to treatment i on day k. Common assumptions were made on the random effects and errors in the repeated-measures model: the b_j's have mean 0 and variance σ_b^2 and are independent of each other and of the e_{ijk}'s; the e_{ijk}'s have mean 0 and e_{ijk}'s with different (i, j) values are independent of each other.

Various covariance structures were selected for modeling the correlations between the response values on different days for the same bird j with treatment i, including independence, compound symmetry, autoregressive (1), autoregressive moving-average (ARMA) (1, 1), and Toeplitz. The Akaike information criterion was used to select the best correlation structure, and ARMA (1,1) was the best for both TS and FV. The results reported for TS and FV are from fitting the mixed linear model with this correlation structure. We tested the hypothesis of no effect of time and no influence of treatment levels over time on the response variables evaluated. The Tukey-Kramer method was used for all pairwise comparisons between the treatments, and a P-value of 0.05 or less indicates a significant difference between two treatments. Unless otherwise specified, results for each treatment are presented as a least-squares mean $\pm$ the standard error of the mean (mean $\pm$ SE).

Results

Daily time spent at the feeder (TS)

There was statistical evidence of the treatment $\times$ day interaction for TS ($F_{12,822} = 2.07; P = 0.02$). The effect of experimental days on TS was also significant ($F_{4,707} =$

$5.02; P < 0.01$). Nonetheless, there were few changes in the TS pattern over time by the group (Figure 3.2). Thus, the main effect of feeder space was elucidated. Significant differences were detected among the feeder space treatments regarding TS ($F_{3,184} = 6.97; P < 0.01$) in that TS decreased with declining feeder space. However, no significant difference was found among the 12.0, 9.5, and 8.5 cm/hen treatments (293 ± 10, 286 ± 10, and 281 ± 10 min/d, respectively) and between the 8.5 and 6.5 cm/hen treatments ($P > 0.05$; Table 2.2). In comparison, TS at both 12.0 and 9.5 cm/hen was significantly higher ($P < 0.05$) than TS at 6.5 cm/hen (269 ± 10 min/d). As shown by the data in Figure 3.3, there existed considerable individual variations in TS among the 60 hens. The coefficient of variation (CV) of TS ranged from 28.0% to 32.1% for the four feeder spaces tested.

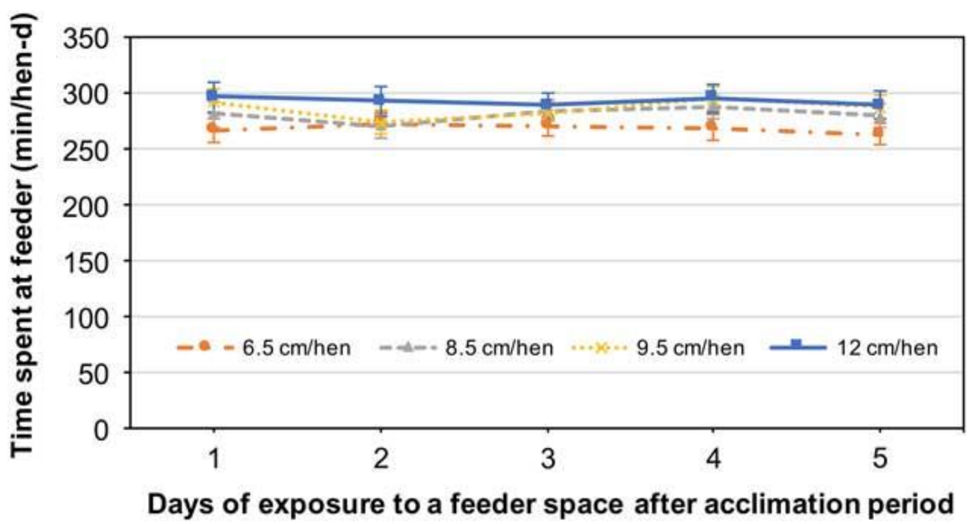

Figure 3.2 *Daily time spent at the feeder (mean $\pm$ SE, min/hen-d) by the laying hens over 5 consecutive days after the acclimation period when subjected to different feeder spaces.*

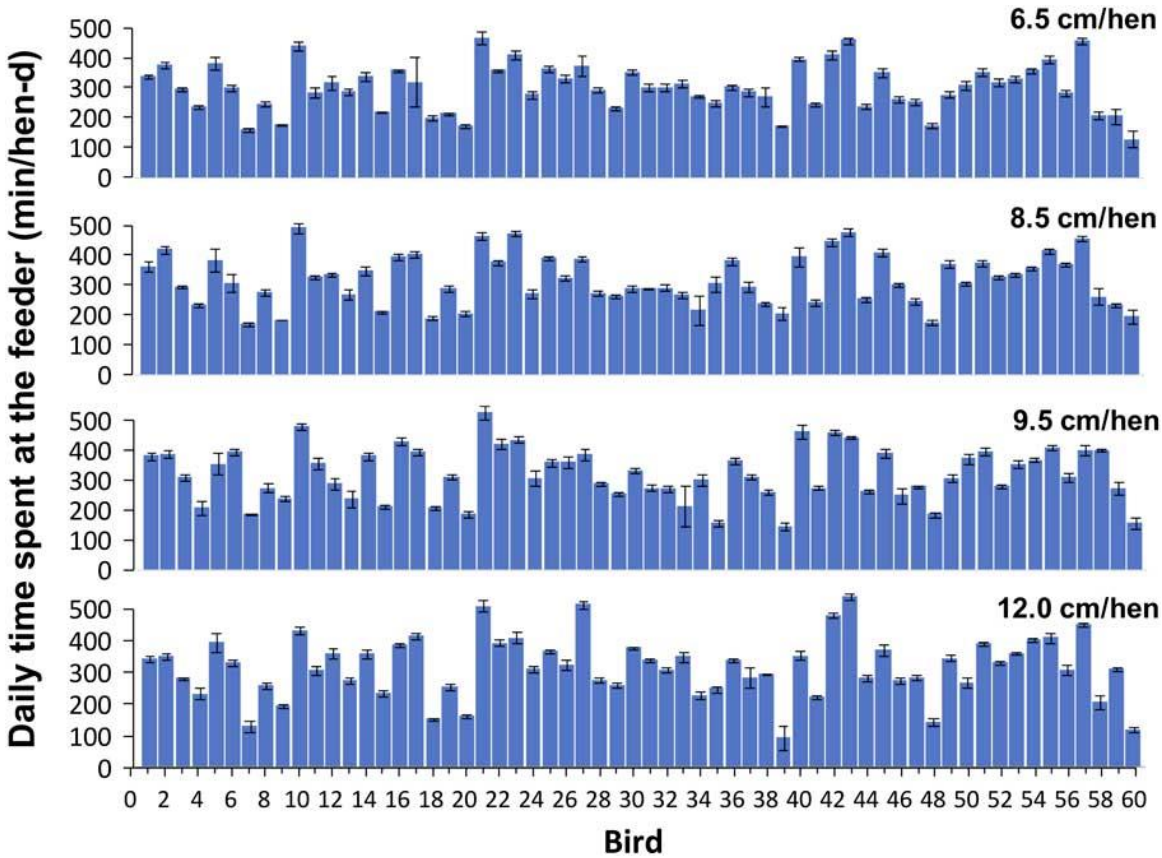

Figure 3.3 *Variation in daily time spent at feeder (mean ± SE, min/hen-d) among the 60 individual hens for a given feeder space (6.5, 8.5, 9.5, or 12.0 cm/hen from top to bottom).*

Table 3.2 *Feeding behavior and production characteristics (least squares means) of 60 laying hens (W-36) in the enriched colony housing (ECH) with different feeder space allocations*

	Feeder spaces (cm/hen)						
Responses	12.0	9.5	8.5	6.5	RSD	$F_{NDF,DDF}$	*P*-value
Time spent at feeder (min/hen-d)	293 ^{a}	286 ^{a}	281 ^{ab}	269 ^{b}	5.3*	$F_{3,184} = 6.97$	<0.01
Frequency of feeder visit (visits/hen-d)	102 ^{a}	92 ^{b}	94 ^{bc}	99 ^{ac}	1.9*	$F_{3,179} = 10.35$	<0.01
Max. hen occupancy at feeder (%)	59.0 ^{a}	57.3 ^{ab}	53.3 ^{ab}	53.0 ^{b}	3.2	$F_{3,16} = 4.29$	0.02
Avg. hen occupancy at feeder (%)	31.7^{a}	30.8 ^{ab}	30.3 ^{b}	29.2 ^{c}	0.6	$F_{3,16} = 14.96$	<0.01
Avg. daily water use (g/hen-d)	156	161	158	154	4.6	$F_{3,16} = 2.23$	0.12
Avg. daily feed intake (g/hen-d)	108	109	109	109	3.7	$F_{3,16} = 0.17$	0.91
Hen-Day Egg production (%)	96.0	93.3	96.3	94.7	3.1	$F_{3,16} = 0.95$	0.44

^{a,b,c} For each response variable, the row means with different letters are significantly different at $P < 0.05$.

* The repeated-measures analysis considers each individual hen. Therefore, the residual standard deviation (RSD) presented here is the RSD from the repeated-measures analysis divided by $\sqrt{n}(n = 60)$ to be comparable with the RSD from the one-way ANOVA results.

NDF = Numerator degree of freedom, DDF = Denominator degree of freedom

Daily frequency of visits to feeder (FV)

There was no evidence of the treatment × day interaction ($F_{12,820} = 1.50; P = 0.12$) or day effect ($F_{4,706} = 1.17; P = 0.32$) regarding FV. However, significant differences in FV were detected among the treatments ($F_{3,179} = 10.35; P < 0.01$). The hens showed more visits to the feeder (102 ± 3 visits/d) in the 12.0 cm/hen treatment than in the 9.5 and 8.5 cm/hen treatments (92 ± 3 and 94 ± 3 visits/d, respectively) ($P < 0.05$), and more visits to the feeder (99 ± 3 visits/d) in the 6.5 cm/hen treatment than in the 9.5 cm/hen treatment. No difference was detected ($P > 0.05$) in FV between 9.5 and 8.5 cm/hen, between 6.5 and 8.5 cm/hen, and between 6.5 and 12 cm/hen treatments. FV showed a nonlinear pattern with feeder space in that the two highest FV values occurred at the smallest and largest feeder spaces (6.5 and 12 cm/hen). Like TS, there existed considerable variations in FV among the individual hens (Figure 3.4). The CV of FV ranged from 26.5% to 27.8% for the feeder spaces tested.

Hens feeding simultaneously

Significant differences were detected among the treatments regarding MPB ($F_{3,16} = 4.29; P = 0.02$) and APB ($F_{3,16} = 14.96; P < 0.01$). APB was observed to be $31.7 \pm 0.3\%$ at 12.0 cm/hen and decreased to $29.2 \pm 0.3\%$ at 6.5 cm/hen. Similarly, MPB was $59.0 \pm 1.4\%$ at 12.0 cm/hen and decreased to $53.0 \pm 1.4\%$ at 6.5 cm/hen. However, the only statistical difference detected in MPB was between 6.5 and 12.0 cm/hen ($P < 0.05$). On the other hand, statistical difference in APB was found between 6.5 cm/hen and all other feeder spaces tested and between 8.5 and 12.0 cm/hen ($P < 0.05$).

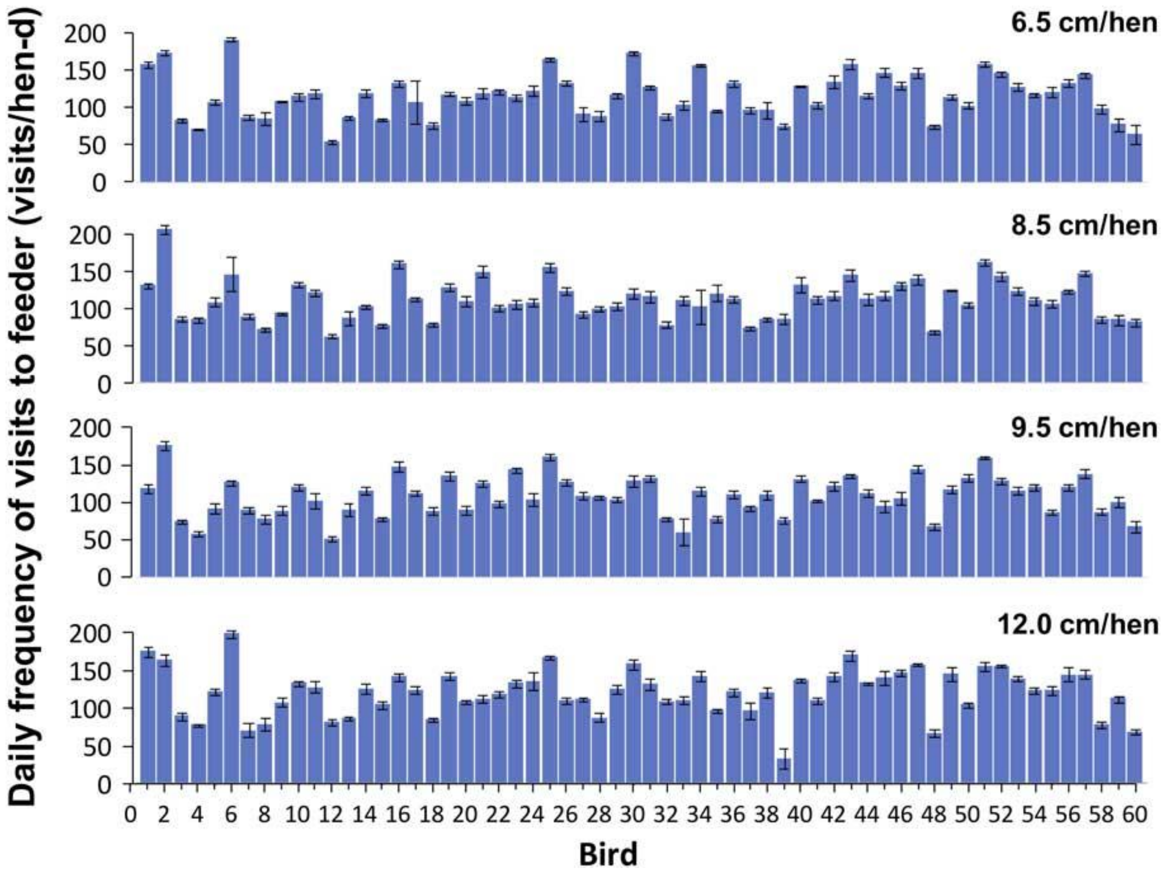

Figure 3.4 *Variation in daily frequency of visits to feeder (mean ± SE, visits/hen-d) among the 60 individual hens for a given feeder space (6.5, 8.5, 9.5, and 12.0 cm/hen from top to bottom).*

The total available feeder space in the colony at 12.0, 9.5, 8.5, and 6.5 cm/hen was, respectively, 7.2, 5.7, 5.1, and 3.9 m. As the feeder space availability changed, the density of birds at the feeder changed and consequently the space between birds changed. Here, the maximum and average density of birds at the feeder (hens/m) for each feeder space tested was determined by dividing respectively the maximum and average number of birds feeding simultaneously by the available total feeder space of the treatment tested. At 12.0 cm/hen, the maximum and average density of hens was 5 hens/m (20.3 cm/hen) and 3 hens/m (37.9 cm/hen). At 9.5 cm/hen, the maximum and average density was 6 hens/m (16.6 cm/hen) and 3 hens/m (30.8 cm/hen). At 8.5 cm/hen, the maximum and average density was 6 hens/m (15.9 cm/hen) and 4 hens/m (28.1 cm/hen). Finally, at 6.5 cm/hen, the maximum and average density was 8 hens/m (12.3 cm/hen) and 4 hens/m (22.3 cm/hen).

Simultaneous occupancy of hens at the feeder, expressed as APB and MPB and pooled over the 5 experimental days by hour, is shown in Figure 3.5. As depicted by the data, the dynamics of feeder usage was similar for all treatments. The percentage of hens feeding simultaneously increased during the day. In general, use of the feeders was relatively mild in the morning (05:00-09:00h; MPB varied from $43.3 \pm 0.6\%$ to $46.7 \pm 3.1\%$), moderate from late morning to afternoon (09:00-16:00h; MPB varied from 49.5 ± 1.6 to $54.5 \pm 0.9\%$), and relatively intense in the late afternoon/evening (16:00 to 21:00h; MPB varied from 52.3 ± 0.4 to $58.0 \pm 1.4\%$). This pattern was consistent in all the treatments, and statistical difference ($P < 0.05$) was observed for the simultaneous occupancy among the three periods for all the treatments tested.

Production performance

Despite the significant differences detected for the behavioral responses (TS, FV, MPB, and APB) among some of the treatments, no treatment effects for the feeder spaces tested were detected on FI ($F_{3,16} = 0.17; P = 0.91$), WU ($F_{3,16} = 2.23; P = 0.12$), and HDEP ($F_{3,16} = 0.95; P = 0.44$) (Table 2). No mortality was experienced during the experiment period.

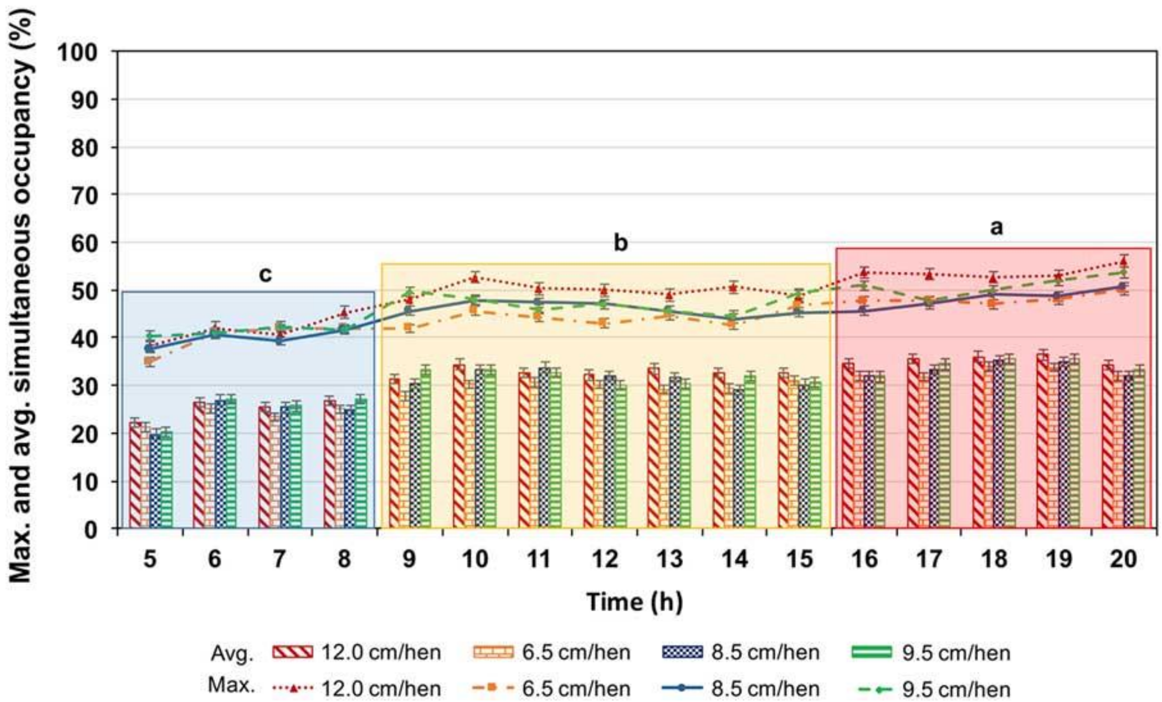

Figure 3.5 *Daily patterns of maximum (mean ± SE, lines) and average (mean ± SE, bars) simultaneous occupancy or feeding of the hens for a given feeder space (6.5, 8.5, 9.5 or 12.0 cm/hen). The colored boxes represent the simultaneous occupancy at feeder in the morning (mild occupation from 0500 to 0900 h – blue box), late morning/afternoon (moderate occupation from 0900 to 1600 h – yellow box), and late afternoon/evening (intense occupation from 1600 to 2100 h – red box). Boxes with different letters are significantly different at P<0.05 for all the treatments tested.*

Discussion

Due to limited resources and to allow for video recording for system performance verification, the RFID system was only installed in the top tier of the ECH module. Consequently, the group-based production performance was not replicated other than repeated measures over the experiment period. Besides, the number of hens involved was rather small. Hence, the performance results should be treated with caution; further validation involving replications and more hens is prudent before a solid conclusion is reached.

Impact of feeder space on feeding behavior

Impact on TS.

While there was an overall treatment effect of the feeder space on TS ($P < 0.01$), no treatment effect was detected between 12.0 and 9.5 cm/hen and between 8.5 cm/hen and any other feeder space tested. Thogerson *et al*. (2009b) evaluated the effect of feeder space (5.8, 7.1, 8.4, 9.7, 10.9, and 12.2 cm/hen) on time spent at the feeder in conventional cages by changing the hen stocking density and found that hens with reduced feeder space spent less time feeding; however, no significant difference ($P > 0.05$) was found in TS between 12.2 and 10.9 cm/hen and between 10.9 and 9.7 cm/hen.

Impact on FV.

The current study revealed a nonlinear relationship between FV and feeder space. This relationship might be explained by noting that when 12.0 cm/hen feeder space was provided, the hens had plenty of space and could use it at their will. In this case, the hens had more opportunities to perform foraging activities in the feeder area. When feeder space was reduced to the most restricted level of 6.5 cm/hen, TS was the lowest but FV was the second highest (99 ± 3 visits/d) among all the treatments. This outcome might have occurred because with less space, the birds had to take more turns to acquire the needed daily feed intake.

The motivations for the two high FV values at the largest and smallest feeder spaces (12.0 vs. 6.5 cm/hen) were presumably different – one for more of exercising leisure behavior versus the other for acquiring necessary feed intake. Seeing the feed but being unable to access it was shown to increase competition (Banks *et al.*, 1979; Meunier-Salaün and Faure, 1984), and the increased FV for the most restricted feeder space seems consistent with such a behavior (although competition behavior was not quantified in the current study). The FV values at 8.5 and 9.5 cm/hen were not significantly different ($P > 0.05$), albeit being significantly lower than FV at 12.0 cm/hen ($P < 0.05$). When evaluating feeding behavior of laying hens under different stocking densities in a conventional cage, Cook *et al*. (2006) reported frequency of feeder visits (mean $\pm$ SE) ranging from 117 ± 22 to 181 ± 22 visits per cage of six hens. In that study, feeder space varied from 7.6 to 10.2 cm/hen, resulting from different stocking densities (348, 387, 426, and 465 cm^2/hen). No statistical difference in FV was detected among the treatments ($P = 0.18$), with FV showing a nonlinear trend as well.

Impact on simultaneous feeding.

With the highest MPB of 59.0% and APB of 31.7% at 12.0 cm/hen and insignificantly different MPB of 57.3% and APB of 30.8% at 9.5 cm/hen ($P > 0.05$), the results demonstrated that not all the hens fed simultaneously even though they had sufficient feeder space to do so. The inherent nature of inter-hen variability observed in TS and FV helps to explain why the hens were not feeding simultaneously.

Albentosa *et al.* (2007) observed that even when given a feeder space of 16 cm/hen (resulting from higher space allocation) and unlimited feed in furnished cages, not all birds chose to feed simultaneously. The maximum percentage of hens feeding simultaneously was 83%, 75%, and 80%, respectively, for furnished cages housing 6, 8, and 10 hens. Blatchford and Mench (2014) assessed the utilization of feeder space by hens housed in ECH and found that a maximum of 70% of the birds fed simultaneously. Widowski *et al*. (2017a) found that mean percentage of birds feeding simultaneously was similar (23 to 24%) regardless of cage size or feeder space, with maximum percentage of hens feeding simultaneously not exceeding 63%.

In aviary systems, simultaneous feeding ranged from 34.8 to 65.2% (maximum) and 12.2 to 19.2% (average) when feeder space varied from 3.8 to 10.0 cm/hen (Sirovnik *et al.*, 2018). The low degree of synchronization at the feeder suggests that motivation for feeding is not only based on space availability. For instance, social dominance aspect makes lower-ranking hens avoid feeding near dominant hens (Keeling and Duncan, 1989).

The 6% reduction in MPB from 59% at 12.0 cm/hen to 53% at 6.5 cm/hen translates to four hens less in simultaneous feeding for the 60-hen colony of the current study. This reduction is small relative to a 46% reduction in feeder space. Thogerson *et al*. (2009b) reported that hens responded to reduced feeder space by desynchronizing their feeding behavior. This finding is supported by the APB and MPB results of the current study as the feeder space changed from 12.0 to 6.5 cm/hen.

In terms of hen density at the feeders, when feeder space decreased from 12.0 to 6.5 cm/hen, the average density increased from 3 hens/m (37.9 cm/hen) to 4 hens/m (22.3 cm/hen), and the corresponding maximum density increased from 5 hens/m (20.3 cm/hen) to 8 hens/m (12.3 cm/hen). We observed that the hens adjusted the space between each other as the feeder space availability changed. When more feeder space was provided, the hens increased the space between each other rather than the simultaneous occupancy at the feeders. This behavior reinforces that not all birds fed simultaneously even when 12.0 cm/hen of feeder space was provided, allowing them to do so. However, the inter-hen space became 12.3 cm at the most restrictive feeder space of 6.5 cm/hen.

The highest feeding activities during the last part of the photoperiod agreed with the results reported in the literature. In evaluating the effect of feeder space allowance (8.9 vs. 12.8 cm/hen) by changing stocking density and cage size on laying hens' behavior in furnished cages, Widowski *et al*. (2017a) observed feeding activities of the hens in 5 different feeding times and found a higher percentage of birds feeding at 17:00h ($35.3 \pm 0.1\%$). Blatchford and

Mench (2014) observed that hens housed in an enriched colony had a small peak in synchronization of feeding at 07:00h and a large peak between 18:00h and 19:00h.

The increased feeding activities of poultry in the later part of the day reflect their anticipatory feeding behavior before the lights go off (dark under natural light conditions) and no feeding activities at night (8-10 hr). This type of feeding behavior had also been reported in domestic fowls (Savory *et al*., 1978; Savory, 1980). The calcium consumed in the evening and stored in the gut would be used to supplement the calcium from the medullary bone during eggshell formation (Graveland and Berends, 1997).

Impact of feeder space on production performance

WU and FI were not affected by the feeder space treatments in this study. The results agreed with some studies in the literature (Cook *et al*., 2006; Diarra and Devi, 2014; Widowski *et al*., 2017b), but conflicted with others that reported effect of feeder space on feed disappearance (Davami *et al*., 1987; Thogerson *et al.*, 2009a; Sirovnik *et al*., 2018). However, when unaccounted for, feed wastage could have led to inaccurate outcome on actual feed intake – the reason for using the term “feed disappearance” vs. “feed intake.”

When given larger feeder space and lower density, birds are allowed to perform non-feeding behavior such as observations of the surrounding, leading to feed wastage (Sirovnik *et al*., 2018).Visual observations during daily routine inspection of the hens noted that they were not always feeding while physically present at the feeders. This non-feeding behavior was also reported by Ma *et al*. (2015), which assessed W-36 laying hens’ choices for light intensity. The authors observed that the overall time spent at the feeder per day was 4.4 ± 0.1 hours; however, the hens spent only 3.4 ± 0.1 hours actually feeding. Li *et al*. (1991) proposed that the long eating time found in a study to evaluate the diurnal variation in heat production related to physical activities in laying hens was due to leisure-eating and food-pecking. These outcomes suggest that feeders have other enriching functions besides feeding.

The feeder spaces tested in this study did not influence HDEP. This result agreed with the results reported in some studies (Thogerson *et al*., 2009a; Sirovnik *et al*., 2018; Widowski *et al*., 2017b), but conflicted with others that reported significant effect of feeder space on egg production in conventional cages or floor pens (Garner *et al*., 2012; Diarra and Devi, 2014). These differences might have arisen from different hen breeds, housing systems, and the experimental conditions used. Specifically, the feeder space ranged from 4.8 to 12.8 cm/hen in the case of the conventional system with white leghorns, and 5.6 to 16.8 cm/hen for the floor pens with Shaver brown hens. The minimum feeder space (4.8 or 5.6 cm/hen) in both cases was smaller than the one (6.5 cm/hen) used in the current study involving W-36 white leghorn hens.

Conclusions

Laying hens (W-36 breed) in the enriched colony housing showed similar feeding behaviors when provided a feeder space of 12.0 or 9.5 cm/hen. Albeit presence of ample spare feeder space, as in the case of 12.0 cm/hen regimen, the hens did not exhibit the behavior of all feeding simultaneously. Hens provided with the 6.5 cm/hen feeder space spent less time at the feeder than with the 9.5 or 12.0 cm/hen feeder space and had a lower maximum number of hens feeding simultaneously than in the 12.0 cm/hen feeder space.

However, no treatment effects (12.0, 9.5, 8.5, or 6.5 cm/hen) were observed on the hen’s production performance responses of daily feed intake, water intake, and rate of lay, although the number of hens involved was quite small. Nonetheless, it is worth noting that lower feeder space may lead to aggression or frustration which was not quantified in the current study. It would be prudent to examine long-term impacts of feeder space on laying hen’s welfare, especially for the feeder spaces that do not impair production performance of the hen in alternative housing systems.

Acknowledgements

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Declaration of interest

The authors declare no conflicts of interest.

Ethics statement

The experimental design and procedures were previously approved by the Iowa State University Institutional Animal Care and Use Committee – IACUC (Log # 6-15-8038-G).

Software and data repository resources

Data were not deposited in an official repository. Access to data can be requested via the corresponding author.

References

Albentosa MJ, Cooper JJ, Luddem T, Redgate SE, Elson HA and Walker AW 2007. Evaluation of the effects of cage height and stocking density on the behaviour of laying hens in furnished cages. British Poultry Science 48, 1–11.

American Humane Certified 2017a. Animal Welfare Standards for Laying Hens- Cage Free.

Retrieved on 23 August 2017, from http://www.humaneheartland.org/our-standards.

American Humane Certified 2017b. Animal Welfare Standards for Laying Hens- Enriched Colony Housing. Retrieved on 23 August 2017, from http://www.humaneheartland.org/our-standards.

Appleby MC 2004. What causes crowding? Effects of space, facilities and group size on behaviour, with particular reference to furnished cages for hens. Animal Welfare 13, 313–320.

Banks EM, Wood-Gush DGM, Hughes BO and Mankovich NJ 1979. Social rank and priority of access to resources in domestic fowl. Behavioural Processes 4, 197–209.

Blatchford RA and Mench JA 2014. The utilization of feeder space by hens housed in enriched colony cages. Poultry Science 93, 71 (Abstr.).

Campbell DLM, Hinch GN, Dyall TR, Warin L, Little BA and Lee C 2017. Outdoor stocking density in free-range laying hens: radio-frequency identification of impacts on range use. animal 11, 121–130.

Cook R. N., Xin H. and Nettleton D. 2006. Effects of cage stocking density on feeding behaviors of group-housed laying hens. Transactions of the ASABE 49, 187–192.

Davami A, Wineland MJ, Jones WT, Ilardi RL and Peterson RA 1987. Effects of population size, floor space, and feeder space upon productive performance, external appearance, and plasma corticosterone concentration of laying hens. Poultry Science 66, 251–257.

Diarra SS and Devi A 2014. Response of Shaver Brown Laying Hens to Different Feeding Space Allowances. International Journal of Poultry Science 13, 714–717.

European Commission 1999. Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens. Official Journal of the European Communities no. L 203, 53–57.

Garner JP, Kiess AS, Mench JA, Newberry RC and Hester PY 2012. The effect of cage and house design on egg production and egg weight of White Leghorn hens: An epidemiological study. Poultry Science 91, 1522–1535.

Graveland J and Berends AE 1997. Timing of the calcium intake and effect of calcium deficiency on behaviour and egg laying in captive great tits, Parus major. Physiological Zoology 70, 74–84.

Hartcher KM, Hickey KA, Hemsworth PH, Cronin GM, Wilkinson SJ and Singh M 2016. Relationships between range access as monitored by radio frequency identification technology, fearfulness, and plumage damage in free-range laying hens. animal 10, 847–853.

Humane Farm Animal Care (HFAC) 2017. HFAC Standards for Production of Egg Laying

Hens. Retrieved on 23 August 2017, from http://certifiedhumane.org/wp-content/uploads/Std17.Layers.1A-2.pdf.

Keeling LJ and Duncan IJH 1989. Inter-individual distances and orientation in laying hens housed in groups of three in two different-sized enclosures. Applied Animal Behaviour Science 24, 325–342.

Li Y, Ito T and Yamamoto S 1991. Diurnal variation in heat production related to some physical activities in laying hens. British Poultry Science 32, 821–827.

Li L, Zhao Y, Oliveira J, Verhoijsen W, Liu K and Xin H 2017. A UHF RFID System for Studying Individual Feeding and Nesting Behaviors of Group-Housed Laying Hens. Transactions of the ASABE 60, 1337–1347.

Ma H, Xin H, Zhao Y, Li B, Shepherd TA and Alvarez I 2016. Assessment of lighting needs by W-36 laying hens via preference test. animal 10, 671–680.

Mench JA and Blatchford RA 2014. Determination of space use by laying hens using kinematic analysis. Poultry Science 93, 794–798.

Meunier-Salaün MC and Faure JM 1984. On the feeding and social behaviour of the laying hen. Applied Animal Behaviour Science 13, 129–141.

Nakarmi A, Tang L and Xin H 2014. Automated Tracking and Behavior Quantification of Laying Hens Using 3D Computer Vision and Radio Frequency Identification Technologies. Transactions of the ASABE 57, 1455–1472.

National Farm Animal Care Council (NFACC) 2017. Code of practice for the care and handling of pullets and laying hens. Ottawa, Ontario, Canada.

Ringgenberg N, Fröhlich EKFF, Harlander-Matauschek A, Toscano MJ, Würbel H and Roth BA 2015. Effects of variation in nest curtain design on pre-laying behaviour of domestic hens. Applied Animal Behaviour Science 170, 34–43.

Sales GT, Green AR, Gates RS, Brown-Brandl TM and Eigenberg RA 2015. Quantifying detection performance of a passive low-frequency RFID system in an environmental preference chamber for laying hens. Computers and Electronics in Agriculture 114, 261–268.

Savory CJ 1980. Diurnal feeding patterns in domestic fowls: A review. Applied Animal Ethology 6, 71–82.

Savory CJ, Wood-Gush DGM and Duncan IJH 1978. Feeding behaviour in a population of domestic fowls in the wild. Applied Animal Ethology 4, 13–27.

Sirovnik J, Würbel H and Toscano MJ 2018. Feeder space affects access to the feeder, aggression, and feed conversion in laying hens in an aviary system. Applied Animal Behaviour Science 198, 75–82.

Thogerson CM, Hester PY, Mench JA, Newberry RC, Okura CM, Pajor EA, Talaty PN and Garner JP 2009a. The effect of feeder space allocation on productivity and physiology of Hy-Line W-36 hens housed in conventional cages. Poultry Science 88, 1793–1799.

Thogerson CM, Hester PY, Mench JA, Newberry RC, Pajor EA and Garner JP 2009b. The effect of feeder space allocation on behavior of Hy-Line W-36 hens housed in conventional cages. Poultry Science 88, 1544–1552.

Tu X, Du S, Tang L, Xin H and Wood B 2011. A real-time automated system for monitoring individual feed intake and body weight of group housed turkeys. Computers and Electronics in Agriculture 75, 313–320.

United Egg Producers (UEP) 2017. Animal Husbandry Guidelines for U.S. Egg-Laying Flocks. Retrieved on 23 August 2017, from http://www.unitedegg.org/information/pdf/2017UEP-Animal-Welfare-Complete-Guidelines.pdf.

Widowski TM, Caston LJ, Casey-Trott TM and Hunniford ME 2017a. The effect of space allowance and cage size on laying hens housed in furnished cages, Part II: Behavior at the feeder. Poultry Science 40, 153–165.

Widowski TM, Caston LJ, Hunniford ME, Cooley L and Torrey S 2017b. Effect of space allowance and cage size on laying hens housed in furnished cages, Part I: Performance and well-being. Poultry Science 96, 3805–3815.

Widowski TM, Hemsworth PH, Barnett JL and Rault J-L 2016. Laying hen welfare I. Social environment and space. World's Poultry Science Journal 72, 333–342.

CHAPTER 4. USE OF RFID TECHNOLOGY TO EVALUATE NESTING BEHAVIOR OF INDIVIDUAL LAYING HENS IN AN ENRICHED COLONY HOUSING

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A manuscript submitted to *IJABE*

Abstract

Alternative housing systems for laying hens are increasingly adopted by US egg industry. Information on behavioral and production responses of hens in such systems remains relatively sparse. The primary objective of this paper was to demonstrate that RFID technology can be used to continuously quantify dynamic nesting behaviors of individual laying hens in a 60-hen enriched colony housing (ECH). Results show that hens spent on average 63.7 ± 1.4 min (mean ± SE) in the nest box and made 23.4 ± 0.7 nest visits during a 16-hr daily light period. Time spent in and visits to the nest box during the 6-hr laying period accounted for 56% and 45% of the light-period value, respectively. Maximum nest occupancy was 29.0 ± 0.4%. Three distinct phases of egg production in nest boxes were observed: initial (1.5h), peak (3.2h, egg laying rate of 0.24 ± 0.01 eggs/min), and late (1.3h). The majority (95.1 ± 0.6%) of the daily eggs were laid in the nest box. Considerable variations in nesting behavior among individual hens and day-to-day variations for a given hen were observed. The RFID system will enable researchers to examine impacts of resource allocations on nesting behaviors of laying hens in alternative hen housing.

Keywords. animal welfare; egg production; RFID; alternative hen housing; individual behaviors

Introduction

Productivity of laying hens has improved considerably over the past 60-70 years because of the advancement in dietary nutrition, genetics, disease prevention, controlled production environment, and husbandry equipment. At the same time, welfare standards of hens continue to elevate and some of the criteria (e.g., allocation of enrichments, housing systems, and management practices) are subject to debate [1–4]. Transitioning of egg production systems from conventional cage to alternative housing (e.g., enriched colony, aviary cage-free) is increasingly occurring in various parts of the world, especially in Europe and the United States, to meet animal welfare requirements or legislations [5,6].

Considering that laying hens have strong nesting motivation as a behavioral need [7,8], enriched colony housing (ECH) systems have been developed to accommodate such behavior, thereby improving animal welfare, by providing enclosures such as plastic curtains and turf mat surface in nest boxes [8–11]. Nesting is one of the important behaviors that has been retained during the hen's domestication [12]. It consists of exploration of nest places followed by sitting (the period when the hen sits and actively prepares for the egg laying), also known as pre-laying behavior, and subsequent oviposition [13]. In the absence of appropriate nest location the hen may extend the pre-laying phase by exploring other places, reducing time for sitting [14], or delaying the oviposition [15]. Due to hormonal influences associated with ovulation, oviposition usually occurs in a short time period of the day, and consequently the pre-laying behavior of some hens kept in a group housing will occur simultaneously [16].

Literature has shown that social factors has a great impact on the time spent in the nest [17], that the laying hens have preference on occupied nests [18], and that hens react to the aspect of the nest box, but are inconsistent in their use of a particular one [19]. However, little is known about the dynamics of nesting among hens in the same group, and some questions remain to be addressed. For instance, how does nesting time of the same hen change from one day to the next? How long do hens use nest box each day? How many hens simultaneously occupy the nest box? Answers to these questions would help to understand nesting behavior of hens and their interactions under certain physical conditions, which will be conducive to housing system design to accommodate the biological needs of hens while maximizing utilization of the resources.

The use of automatic measurement or monitoring systems in livestock facilities has provided information to better understand animal well-being. However, the traditional method (e.g., visual analysis) used to assess animal behaviors is dauntingly time-consuming and labor-intensive for addressing behavioral characteristics of individual animals. Radio Frequency Identification (RFID) system has been used to study animal behaviors [20–23]. It consists of a reader with a decoder to interpret the acquired data, scanning antenna, and pre-programmed transponders. RFID transponders can be active when a power source is used, or passive when the transponder is powered by the antenna. RFID systems were used to automatically track laying hen behaviors [24], investigate laying hens' behavior in a preference chamber [25], and evaluate the impacts of outdoor stocking density on the welfare and behavior of free-range laying hens [26]. Several tracking systems were used to study individual laying hens behaviors, and the strengths and weaknesses of each as well as the environment or conditions suitable for using them were discussed [27].

Although some studies have been conducted to evaluate animal behaviors with the aid of RFID, information regarding nesting behaviors of laying hens in enriched colony housing (ECH) is incipient. Such information can build the baseline of such behavior in commercial ECH, and has implication on future advances in the design and allocation of enrichments and/or management practices.

In the first part of a larger experiment that we conducted to assess feeding and nesting behavior of individual laying hens in ECH, one Ultra High Frequency (UHF) RFID system was developed, validated [28] and used to evaluate the impact of feeder space on feeding behavior [29]. The primary objective of the work reported in this paper was to demonstrate that RFID technology can be used to continuously quantify dynamic nesting behaviors of individual laying hens in a group-housing condition. The usefulness of the system was then illustrated by characterizing nesting behavior of Hy-Line W-36 laying hens in an enriched colony housing (ECH), including: daily time spent in the nest box (TS, min/hen-d), daily frequency of visits to the nest box (FV, visits/hen-d), number of visits per egg laid in the nest box (VE, visits/egg), simultaneous occupancy of the nest box (SO, %), oviposition time (OT, hh:mm), oviposition place (OP, % eggs laid in the nest, middle or scratch areas), and nesting association (percentage of nesting time that the hens spend with one another). Variations in nesting behaviors among the individual hens and day-to-day variations for a given hen were also elucidated.

Materials and Methods

The experimental protocol had been approved by the Iowa State University Institutional Animal Care and Use Committee – IACUC (Log # 6-15-8038-G).

Animals and housing

Sixty Hy-Line W-36 (white) laying hens at 21 weeks of age (WOA) were obtained from a commercial farm with enriched colony housing in central Iowa. The pullets (young hens before lay) had been reared in standard rearing cages with the following conditions: 0.61 m long × 0.76 m wide each, 18 birds/cage (258 cm^2/bird), two nipple drinkers, feed trough with available space of 3.4 cm/bird, and galvanized wire-mesh floor. At 17 WOA, the pullets were transferred to the commercial enriched colony hen houses equipped with enriched colony system (AVECH II, Big Dutchman, Vechta, Germany), where they were housed in 60-bird colonies with stocking density of 750 cm^2 floor area/bird.

The top tier of a double-tier ECH module, measuring 3.73 m long × 1.91 m wide × 1.91 m high (Big Dutchman, Vechta, Germany) was used in this experiment (Figure 4.1). The ECH module, located in our research laboratory at Iowa State University, featured a space allocation of 976 cm^2/hen of floor area which included the nest box (85.4 cm^2/hen), scratch area (85.4 cm^2/hen), perches (15.7 cm/hen), nipple drinkers (10 hens/drinker), feeder space (12.3 cm/hen), tier height of 56 cm, manure belt underneath, along with room heating and humidification to maintain the comfortable temperature and humidity. The nest boxes had orange-colored flexible curtains and artificial turf mat. Feeder troughs were on both sides of the colony. Manure was collected on a plastic tarp placed underneath the colony tier and removed once or twice a week. No litter, feed or other substrate was added to scratch pad area.

The hens were fed twice daily at 09:00h and 17:00h (ad libitum) with the same feed as used on the commercial farm where the hens came. A photoperiod of 16-hr light and 8-hr dark (16L:8D) as practiced on the farm was used, with lights on at 05:00 and lights off at 21:00. The range of the light intensity was 13-120 lux across the top tier colony, with the lower intensity (13 lux) near the nest box area and the higher intensity near the scratch pad area (120 lux).

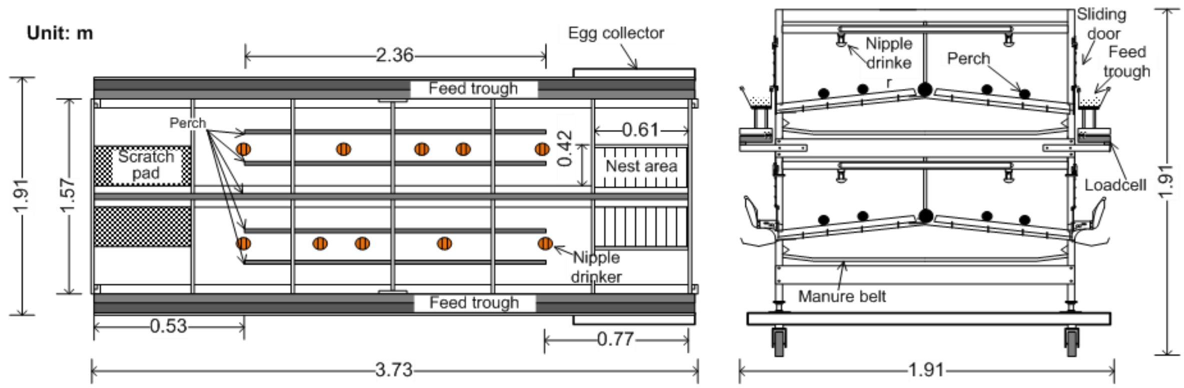

Figure 4.1 *Top-view (left) and side-view (right) schematic drawings of the enriched colony module used in the experiment.*

Eggs laid during the experiment period were manually gathered daily at 17:00h from the egg belts and nest eggs collectors that were made of wooden troughs (0.65m long x 0.30m wide x 0.20m high) situated where the egg belt normally was, covering the nest width (Figure 4.1). Location of eggs was recorded as nest, middle or scratch area. Eggs possibly laid in the space between the nest curtain and the feeder were registered as nest eggs. Similarly, eggs possibly laid in the space between the scratch pad and the feeder were registered as scratch-pad eggs. Eggs were never observed to roll from one section to another. Upon transfer from the commercial farm to the ECH module in the research lab, the hens were given 7-day acclimation. Another 2-day acclimation was given after attachment of the RFID tags to the hen's leg. The data collection lasted 14 days, with the first two days still considered part of the acclimation period. During the acclimation period, feed and water use and egg production were monitored to ensure that the hens were feeding and producing normally.

Instrumentation

The top tier colony was instrumented to monitor real-time feed and water use, record egg production (timing and number), and track the individual hens. Calibrated load-cell scales (Rice Lake RL1040, Rice Lake, WI) were used to continuously (every second) weigh the nest eggs collectors (one load cell per collector) with a maximum measurement error < 0.1% of the total weight measured. This information was used to determine the timing when the nest eggs were deposited in the egg collector. Details on the calibration of the load cells were previously described [28].

Two cameras (IP Pro 3 Megapixel Bullet, DSS-BFR3MP, Backstreet Surveillance, Salt Lake City, UT) were installed on the ceiling above the ECH module and used to record the hen behaviors at 2 frames per second (fps). Video files were stored in 8 terabyte storage (two hard drives) of one NVR system (DSS-NVR5816, Backstreet Surveillance, Salt Lake City, UT).

Air temperature, relative humidity (RH) and carbon dioxide (CO_2) concentration of the hen room were measured and recorded. All sensors were connected to a compact FieldPoint Module, and data recorded using a LabVIEW program (National Instruments Co., Austin, TX, USA). The research lab used an automatically controlled ventilation system that consisted of an environment controller (Varifan ECS-3C, Quebec, Canada), two variable speed exhaust fans (Multifan, Vostermans Ventilation, Bloomington, IL, USA), and a supplemental heating and cooling system. The room temperature was maintained at 23 ± 1 °C (mean ± SE) throughout the experiment.

RFID System

Nesting behavior was evaluated using one UHF RFID system that consisted of four antennas (Square A1030, 30 cm x 30 cm x 0.65 cm thick, TransTech Systems) located on both sides of the nest box, one 4-channel reader (ThingMagic Mercury M6, 865-928 MHz operating frequency, TransTech Systems), 60 individual passive tags (902-928 MHz, PT-103, tie-wrap tag passive Gen 2 UHF, TransTech Systems), and a data acquisition (DAQ) system. The readers were connected to a host computer that processed the tag data through an RJ45 (Registered Jack, 10/100 Base-T Ethernet). The tag protocol was EPCglobal Gen 2 (ISO 18000-6C) with Digital Rights Management (DRM). The data acquisition program was written in C# (C Sharp) based on Application Programming Interface (API) and the data were stored as text files.

One tag was loosely attached to each hen's leg with a zip tie. A hen was registered as nesting when the tagged leg was inside the nest box. The RFID system continuously registered hens inside the nest box, but not if the tagged leg was outside the nest mat while the rest of the body was inside the nest box, which corresponded to broken data. Intermittent brief breaks up to 30 s were considered as part of a nesting event, verified by video as well as previously validated [28].

Measurements and data processing

Load cells were used to determine group-level production of nest eggs and determine the OT. The data were collected every second via a program developed in LabVIEW, and then processed using EXCEL VBA programs. From the 12 days of load cells data collection, days 5 and 11 presented system disconnections with generation of corrupted files and therefore the data from these two days were discarded. The OT was calculated by averaging the time that the first eggs (start time) and last eggs (end time) were registered in the nest eggs collectors. The OP was determined by calculating the average of the daily percentage of eggs laid in the three areas described as nest, middle, and scratch areas.

Data collected with the RFID system consisted of the time (hh:mm:ss:ms) when a specific hen (tag #) was detected nesting. The number of hens detected by the RFID system was compared to and validated by that determined by the video system, i.e., human visual labeling [28]. The overall accuracy of the RFID system relative to the video observation was (mean ± SD) 91.4% ± 1.7% (n = 78). Data analysis and processing were done using R and EXCEL VBA programs to describe the response variables TS, FV, and SO. Data analysis was performed considering two different periods of daily time: 1) Light period (05:00 – 21:00h), and 2) Laying period including the pre-laying phase of nest exploring (05:00 – 11:00h). The laying period was defined after preliminary evaluation of the nest occupancy and egg laying rate data. VE was calculated as ratio of the number of nest visits to eggs laid in the nest box.

Presence of individual hens in the nest box was used to delineate nest box usage of the hens and their nesting association (i.e., percentage of total nesting time when they nested with other hen or hens). The day-to-day consistency in nesting pattern, as measured by the longest duration of stay in the nest box, was assessed for each hen. This longest stay duration was termed ‘main nest visit’. Visits other than the main nest visit were considered random visits, and FV of the hen was compared with the average FV for the laying period to classify as intense (FV of the hen > Average FV), or moderate random visits (FV of the hen ≤ Average FV). Similarly, the average TS for the laying period was used to classify the usage of the nest box as intense (TS of the hen > Average TS) or moderate (TS of the hen ≤ Average FV). The day-to-day variation of the main nest daily visit of individual hens with identified nesting patterns was further classified according to the timing of nest visit.

After evaluation of all individual nest box usage data, nesting patterns were categorized into 5 groups according to the following criteria: 1) clear presence of nesting pattern (when the main nest visit is consistently observed during the laying period on most of the experiment days) with few or no random visits; 2) clear presence of nesting pattern, with moderate use of the nest box and several random visits; 3) clear presence of nesting pattern, with intense usage of the nest box during the laying period, with several random visits; 4) no clear nesting pattern, despite intense usage of the nest box and; and 5) no clear nesting pattern, with moderate usage of the nest box.

Association was considered when two or more birds used the nest box simultaneously during the laying period. A matrix was constructed for the amount of time that each bird was associated with others for each day evaluated. The matrices were symmetric along the diagonal (if bird A nested with bird B, then bird B must have nested with bird A). The diagonal represented the total time that the bird spent in the nest box, and it was used to create the matrix of percentage time spent with each other (the amount of time that bird A spent with bird B divided by the total time that bird A spend in the nest box during the observation day). These matrices were not symmetric about the diagonal (Although bird A and B spent the same time together in the nest box, they individually spent different time in the nest box during the observation day, and consequently their percentage time spent together was different). The association matrix accounted for the number of birds that spent at least X% of their time together inside the nest box in the laying period during Y days, with X ranging from 0 to 100%, and Y ranging from 1 to 12 d.

Statistical analysis

Data were tested for homoscedasticity and normality. The data for TS and FV were square root transformed and analyzed as repeated measures using JMP 13.2.1 (SAS Campus Drive, Cary, NC), following the mixed linear model:

$$y_{jk}=\mu+b_j+\gamma_k+e_{jk} \quad (1)$$

where y_{jk} is the response (TS or FV) value on day k for bird j; μ is the overall mean effect; b_j is the random effect of bird j; γ_k is the fixed effect of experimental day k; and e_{jk} is the random error associated with bird j on day k. Common assumptions were made on the random effects and errors in the repeated-measures model: the b_j*'s* have mean of 0 and variance of σ_b^2, are independent of each other, and of the e_{jk}*'s*; the e_{jk}*'s* have mean of 0 and e_{jk}*'s* with different j values being independent of each other.

Results of TS and FV were obtained from analysis of variance by fitting the mixed linear model (1) with the auto regressive correlation structure. The hypothesis tested was that there was no time effect on the response variables. Data of SO, OT, OP, VE, individual nesting pattern and nesting association were pooled over the experiment period, and descriptive statistics were provided. P-value of 0.05 or lower was considered significant. Unless otherwise specified, results are presented as a least-squares mean ± standard error (mean ± SE).

Results

Daily time spent in nest box (TS)

There was no evidence of time (day) effect on TS for the light period ($P = 0.32$, $F_{1,170.9} = 0.85$) or laying period ($P = 0.13$, $F_{1,179.8} = 2.29$). Therefore, data from all days were pooled to analyze variability in TS among the individual hens. The daily time spent in the nest box by each hen was determined and individual variation in TS is shown in Figure 4.2.

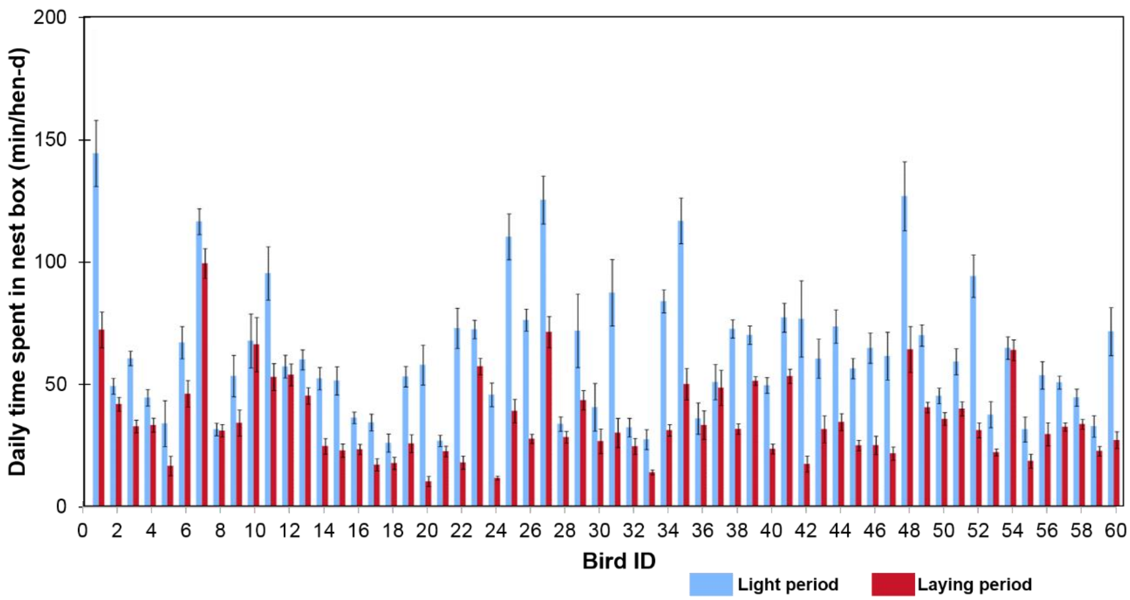

Figure 4.2 *Variation in daily time spent in the nest box (mean ± SE, min/hen-d) by individual laying hens in an enriched colony housing. The blue bars represent time spent during the light period (05:00 to 21:00h) while the red bars represent time spent during the laying period (05:00 to 11:00h).*

TS was 63.7 ± 1.4 and 35.9 ± 0.9 min/hen-d during the light and laying period, respectively. As depicted in Figure 4.2, there were considerable inter-hen variations in daily time spent in the nest box. Expressing the variability in terms of coefficient of variation (CV), the values were 56.4% and 61.7% for the light and laying period, respectively. It was possible to identify the hens that used the nest boxes habitually during the laying period (05:00 to 11:00h) and those that kept visiting the nest boxes even after the laying period.

In this experiment, it was observed that most of the hens spent extra time in the nest box after the laying period. Specifically, 26.7% of them used the nest box predominantly during the laying period, with no significant difference in TS between the laying and light periods (Hens # 2, 5, 8, 9, 10, 12, 18, 21, 28, 29, 30, 32, 36, 37, 54, and 55), whereas 73.3% of the hens used the nest box during the laying period and kept using the nest box after the laying period.

Daily frequency of visits to nest box (FV)

All exploring and laying visits to the nest box by the hens were registered, from which FV could be quantified for each of the 60 hens. The daily FV of individual hens is presented in Figure 4.3.

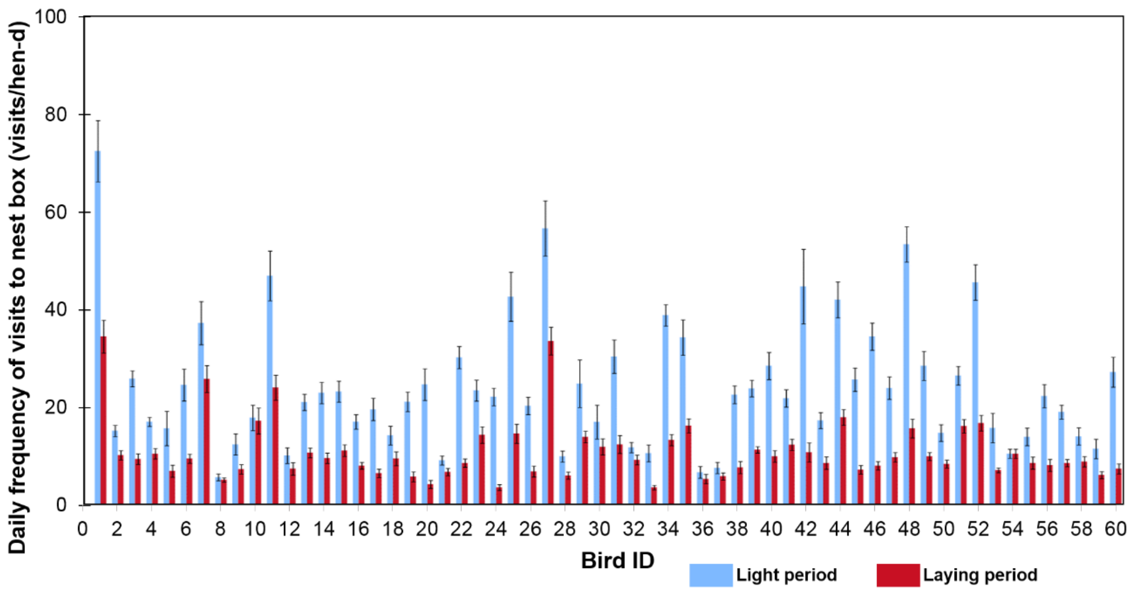

Figure 4.3 *Variation in daily frequency of visits to nest box (mean ± SE, visits/hen-d) by individual laying hens in an enriched colony housing. The blue bars represent visit frequency during light period (05:00 to 21:00h) while the red bars represent visit frequency during laying period (05:00 to 11:00h).*

There was no statistical evidence that FV was influenced by time (day) for the light period (P = 0.87, $F_{1,171.7}$ = 0.02) or the laying period (P = 0.59, $F_{1,183.8}$ = 0.13). Therefore, data from all days of monitoring were pooled to analyze variability in the FV among the individual birds. FV was 23.4 ± 0.7 and 10.4 ± 0.3 visits per hen per day for the light and laying period, respectively. CV among the hens in FV was 67.0% and 70.2% for the light and laying period, respectively, showing considerable variability among the individual hens in the ECH.

Quantification of hen-specific number of visits per egg laid in the group nest boxes was not possible with the current setup of the housing and instrumentation systems. The average VE were 25.7 ± 0.8 and 11.4 ± 0.4 visits per egg laid in nest box, for the light and laying periods respectively. In general, use of the nest box was more intensive in the morning (05:00 to 11:00h) or laying period and mild in the rest of the day. The evolution hour-by-hour of the time spent in the nest box (min/hr) and the frequency of visits to nest box (# visits/hr) are depicted in Figure 4.4.

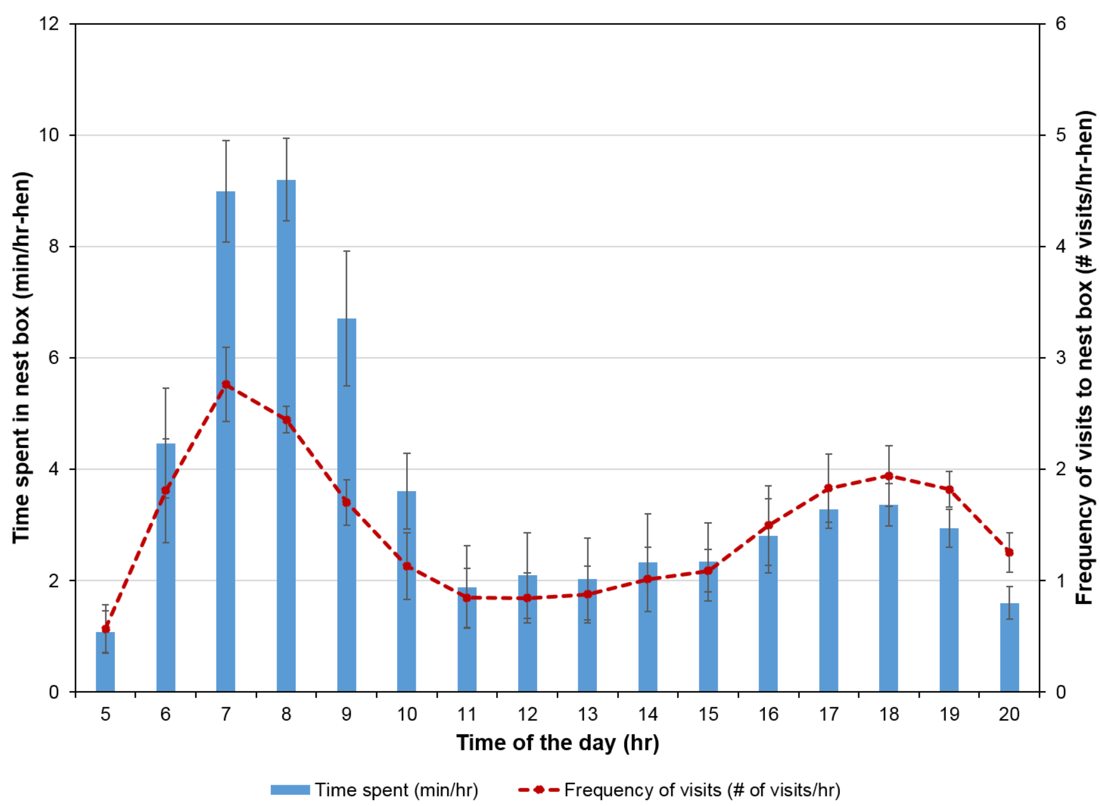

Figure 4.4 *Hourly time spent in nest box (min/hr-hen) and frequency of visits to nest box (visits/hr-hen) by the laying hens during the experiment period (pooled data of 60 hens over 12 d).*

Hourly TS and FV changed considerably during the day. The peaks of hourly TS and FV coincided between 07:00h and 09:00h and followed the same pattern as the diurnal profile of nest box occupancy. They showed a parabolic pattern during the laying period, where hourly TS and FV increased until 08:00h and then decrease until the end of laying period at 11:00h.

Maximum simultaneous occupancy (SO) in the nest box, oviposition time (OT) and place (OP)

SO changed with time throughout the day. The maximum occupancy, 29.0 ± 0.4%, occurred between 07:00h and 09:00h, within 4-hr after the light came on at 05:00h. After this peak time, SO decreased and maintained at low levels until lights off at 21:00h (Figure 4.5).

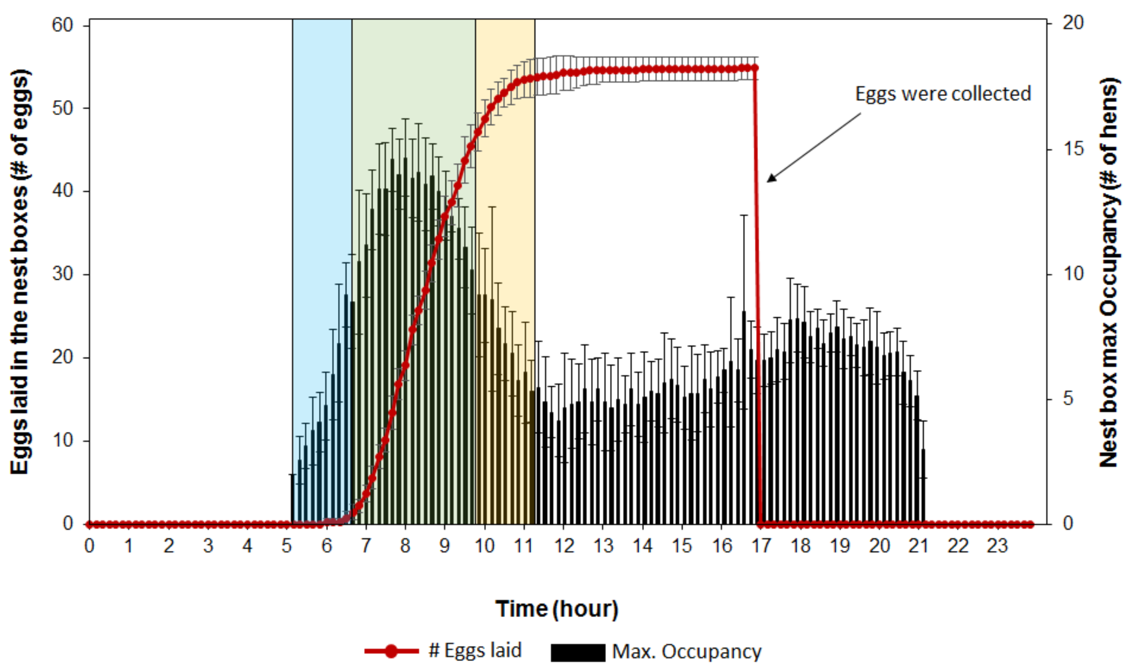

Figure 4.5 *Diurnal profile of nest box maximum occupancy by the laying hens and cumulative registered number of eggs in egg collectors in an enriched colony housing module. The blue, green and yellow rectangles represent, respectively, the initial phase, peak phase, and late phase. Lights came on at 05:00h and went off at 21:00h.*

During OT, three distinct phases of egg production in the nest boxes were observed: 1) Initial phase (no egg laying despite nest visit) – featuring start of nest exploration and increase in nest occupancy, which lasted from 05:00 to 06:30h when the first egg was laid in the nest box (blue rectangle in Figure 4.5); 2) Peak phase – featuring peak egg laying and nest occupancy which lasted from 06:30 to 09:40h when egg laying started decreasing. In this phase, egg laying followed a linear trend with a rate of 0.24 ± 0.01 nest eggs/min (green rectangle in Figure 4.5); 3) Late phase – featuring reduced rate of laying (non-linear trend) and nest occupancy from 09:40h to 11:00h (yellow rectangle in Figure 4.5).

The OP was characterized by nest, middle or scratch eggs. Most of the nest eggs (82.9%) were laid in the nest box between 06:30h and 09:40h, which partially coincided with the period of maximum nest box occupancy (07:00 to 09:00h). As expected, the majority (95.1 ± 0.6%) of the daily eggs were laid in the nest box, while 3.8 ± 0.9% eggs were laid in the scratch area, and only a few (1.1 ± 0.6%) were laid in the middle area.

Patterns in nest box usage and association during nesting

The usage of nest box during laying period differed among the hens. After evaluation of all individual nest box usage data, 5 groups of nesting patterns were established: 1) 15% presented clear nesting pattern with few or no random visits (FV ≤ 10.4 visits/d); 2) 45% presented clear nesting pattern, with moderate nest usage (TS ≤ 35.9 min/d) and several random visits (FV > 10.4 visits/d); 3) 8.3% of the hens presented clear nesting pattern and used the nest box intensively (TS > 35.9 min/d) during the laying period, with several random visits (FV > 10.4 visits/d); 4) 21.7% of the hens presented no clear nesting pattern and had intense use of the nest box (TS > 35.9 min/d); and 5) 10% of the hens presented no clear nesting pattern and had moderate presence in the nest box (TS ≤ 35.9 min/d) (Figure 4.6).

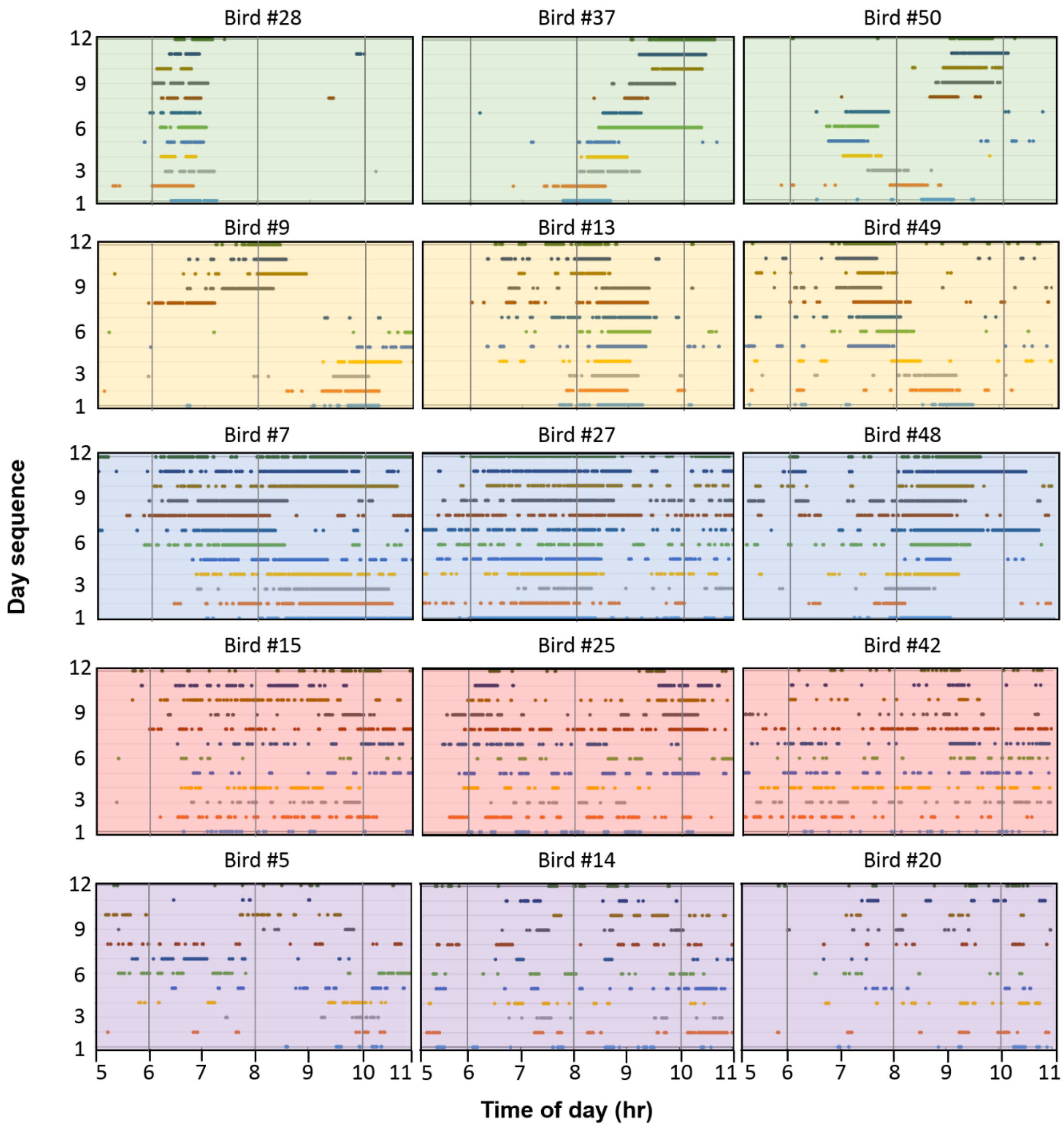

Figure 4.6 *Samples of presence of individual hens in the nest box during laying period (05:00-11:00h) over 12 consecutive days. Each colored rectangle represents one different nesting pattern group: 1) There is clear nesting pattern, with few or no random nest visits (green shade), 2) There is clear nesting pattern, with moderate use of the nest box and several random nest visits (yellow shade), 3) There is clear nesting pattern, with intense use of nest box and several random nest visits (blue shade), 4) There is no clear nesting pattern, with intense use of nest box (red shade), and 5) There is no clear nesting pattern, with moderate or low use of nest box (purple shade).*

The day-to-day variation in nesting behavior of the laying hens was examined by evaluating the dynamic of the prolonged visits time for the same hen over the experiment period. Clear nesting pattern was observed in 68.3% of the hens. Specifically, from the 68.3% hens, 29% visited the nest box daily at the same time (see hen #28 in Figure 4.6), 27% visited the nest box earlier every day (see hen #49 in Figure 4.6), 20% visited the nest box later every day (see hen #37 in Figure 4.6), and 24% presented a mix of earlier and later visits to the nest box (see hen #50 in Figure 4.6).

During the laying period (05:00 – 11:00h), a higher degree of nesting association among the hens occurred more often when the time of nesting together was low, and vice versa. Specifically, 29 hens spent at least 10% of their time together in 9 out of 12 days, 8 hens spent at least 40% of their time together in 5 out of 12 days, and 4 hens spent at least 80% of their time together in one day only (Figure 4.7).

Discussion

Due to limited resources and to allow video recording for system performance verification, the RFID system was only installed in the top tier of the ECH module. Consequently, the group-based variables (OT, OP, VE and SO) were not replicated other than repeated measures over the experiment period. Besides, the number of hens involved and experiment period were rather small. It should be noted that the study did not cover measurements such as aggression or frustration that may arise when nests are crowded. Hence, the results should be treated with caution; further validation involving replications and more hens is prudent before a solid conclusion is reached.

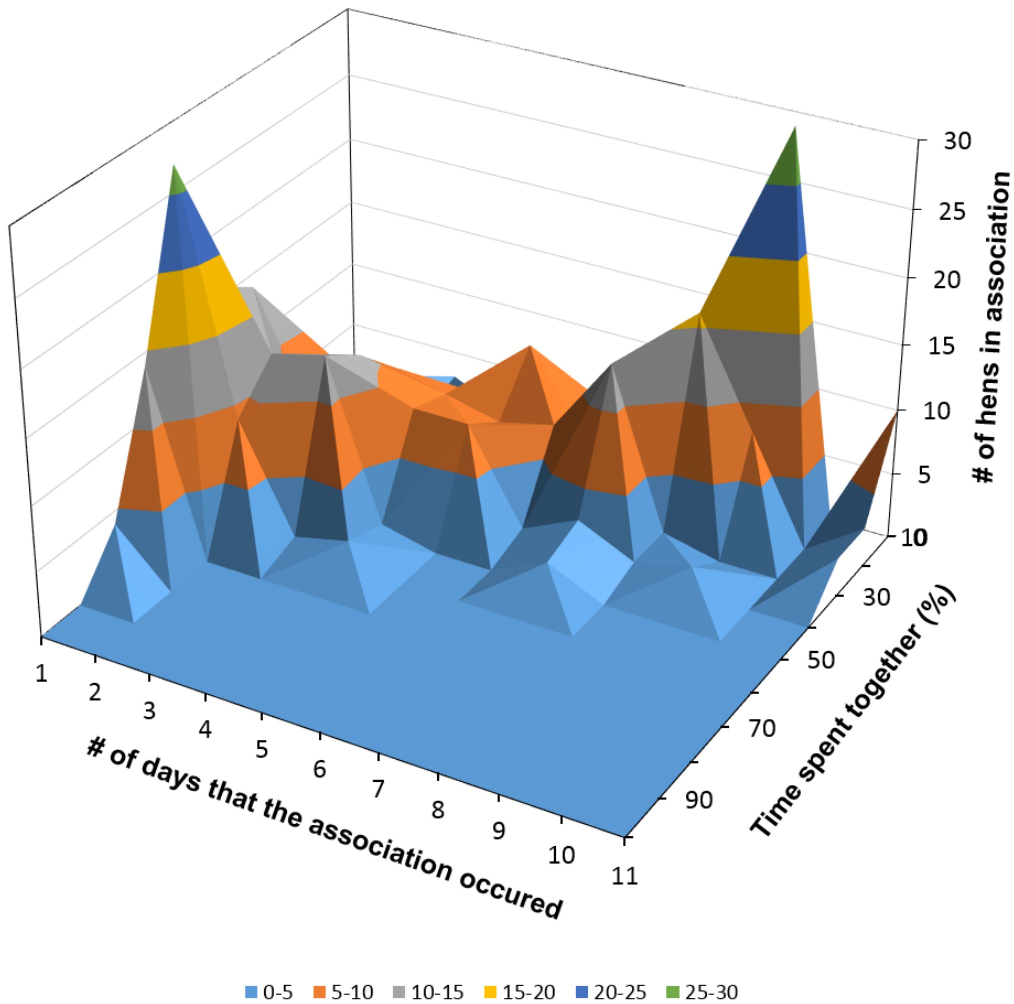

Figure 4.7 *Association of laying hens in nest box during the laying period (05:00 to 11:00h). The association refers to the number of hens (Z axis) that spent their nesting time together (Y axis) for the number of days (X axis) over the 12-day experiment period.*

Use of the RFID technology provided unique individual information that helped to understand the dynamics of nest box usage and nest egg laying, and to identify hens that potentially overused the nest boxes than others. This study, however, could not discern the cause for certain hens to spend more time in the nest. Nevertheless, it may be postulated that social hierarchy played a role in that dominant hens intimidated the subordinate ones, making them delay their oviposition time or simply lay eggs in a different place [30,31]. Alternatively, the subordinate hens could have used the nest boxes not only for laying eggs but as a refuge shelter from the dominant ones [32]. The current study, however, did not quantify social hierarchy of the hens.

TS during the 6-hr laying period (35.9 ± 0.9, min/hen-d) was approximately 56% of the TS during the 16-hr light period (63.7 ± 1.4, min/hen-d). This result paralleled the findings reported previously about the influence of nest-floor slope on nest choices, where it was found that birds could spend 10 to 90 min in the nest when laying an egg [33]. Other studies have reported that hens occupied nest box for approximately 40 min per day [34], 29 min prior to egg laying with extended 6.5 min before leaving the nest box [35]. The use of nest box after laying period is not recommended as the nest mat would be highly prone to excreta deposition that could in turn lead to dirty eggs [36].

The hens were observed to have different individual nesting patterns, and only 26.7% of them used the nest box predominantly during laying period. The overall VE during the light period (25.7 ± 0.8, visits/egg) was approximately twice the value for the laying period (11.4 ± 0.4, visits/egg). High number of nest visit per egg (40.3 ± 11.4) has been observed when testing preferences of nest options in groups of 20 hens [37]. In addition, in an experiment to investigate the effect of nest size on the nesting behavior it was found that the number of visits per egg laid was 34.9 ± 3.7 [38]. The revisit can be explained as the exploratory behavior of the hens [39], or an indication that the enrichments inside the colony were not enough to keep them attracted. It reinforces that nest box area has attractiveness beyond the purpose of laying the egg; however, this practice should be discouraged in order to avoid manure deposition on the nest mat.

The number of hens nesting simultaneously as a function of time during the day showed similar behavior through the experiment period. The visits started right after the lights came on at 05:00h and increased to a peak of 29.0 ± 0.4% occupancy (from 07:00 to 09:00h). After the peak, the nesting synchronization decreased and maintained a minimum level until the lights went off (21:00h). Laying hens have been shown to use nest boxes mostly in the morning, followed by the midday and evening [40]. Our results also agreed with previous findings that when evaluating the influence of nest site on behavior of laying hens the main laying period for hens in commercial aviary systems was between 1 to 4 hr after lights were turned on [41]. The concentration of hens in the nest box during the peak time may be explained by the phenomenon of gregarious nesting, also known as the preference for occupied nests [18,42], attributing this behavior to the sense of protection [38].

This experiment found that 83% of the nest eggs were laid proportionally in time within the period between 1 hr and 30 min and 4 hr and 40 min after lights-on (05:00h), with an average laying rate of 0.24 ± 0.01 eggs/min in the nest box. This pattern coincided mostly with the peak of simultaneous nest box occupancy period (from 07:00 to 09:00h). Previous research has shown a nonlinear pattern of egg laying with a peak of eggs laid around 3.5 h [43] or 3.5-4.5 h [44] after lights-on. In an investigation of the nest use and patterns of egg laying of 4 different strains of laying hens in aviary system, it was found that all strains presented a peak of nest usage at 08:00h (3-hr after the lights came on) [40]. In that study, brown hens (Hy-Line and Bovan) showed similar behavior with 85% of the nest eggs laid from 06:00 to 10:00h, while the white hens (Hy-Line W36 and Dekalb White) laid about 55% of their daily nest eggs during the same time period.

The three distinct phases egg production in nest boxes indicate that the hens were exposed to a less competitive nest box during the third phase, which suggests that oviposition delay could be motivated by stress and its impact on hen's physiology [31]. It is important to understand the motivations of oviposition delay, especially considering that such delay may cause abnormal eggshell formation [31], impair reproduction by cessation of egg laying [45], and trigger floor eggs in aviary or free-range housing systems [46].

It takes about 24-hr after ovulation to complete the formation of an egg, which is divided into the following: 4 hr for addition of the albumen layers to the yolk as it passes along the oviduct; 5 hr for the membranes formation in the tubular isthmus and absorption of water and salts in the shell gland; and 15 to 16 hr for the shell calcification [47,48]. However, for the hens with identified nesting patterns, the day-to-day pattern of oviposition time varied considerably, and hens were classified into four different groups: 1) hens that visited the nest box every day at the same time, 2) each day earlier, 3) each day later or 4) mixed periods (earlier and later). While this experiment could not identify if a specific hen indeed laid an egg when inside the nest box, this observation suggests that the 24-hr period between egg laying is not consistent among individuals and need further investigation. A few hens did not visit the nest box during laying period on some days. This behavior could be related to physiology when considering that the hens pause laying between clutches [49,50], social factors such as presence of dominant hens [30], lack of interest in delaying oviposition to a less competitive time [31], or specific preference of the scratch and perch areas over the nest box. The rearing environment affects nest use, and individual laying hens might perceive nest sites differently [51].

Although various degrees of association during nesting were detected, it was not sufficient to conclude that the hens had social preferences, or that their oviposition cycle was synchronized. Social preferences of laying hens expressed by close active and resting proximities were not found in a group of 15 hens over a period of 8 weeks based on three 15-min scanning periods (one day per week for active space use, and three days per week for roosting association) [52].

As expected, most of the eggs were laid in the nest box area (95.1 ± 0.6%), while 3.8 ± 0.9% of the eggs were laid in the scratch area and even a lower proportion (1.1 ± 0.6%) of eggs were laid in the middle open area. In furnished cages, similar results were found where 91.7% of the total eggs were laid in the nest box, 7.2% in the scratch area, and 1.1% in the perches area [43]. In a recent study with aviary systems, the OP was quite similar to the findings in ECH, where 90.5 – 94.9% of the eggs were laid in the nest boxes, and 2.3 – 4.4% of the eggs were laid on the litter [40].

Conclusions

This study demonstrates that the validated UHF-RFID system can be successfully used to continuously and automatically monitor nesting behavior of individual hens kept in a group. Application of the RFID system with an enriched colony housing (ECH) revealed significant variability in nesting behavior among individual laying hens. The degree of temporal variation in nesting behavior also varies considerably among individual hens in the ECH. The RFID system will enable researchers to examine the impacts of resource allocations on nesting behaviors of laying hens, which may in turn help guiding design of alternative hen housing systems.

Author Contributions

Conceptualization, J.O., H.X., and Y.Z.; Methodology, J.O., H.X., and Y.Z.; Software, J.O. and K.W.; Validation, J.O.; Formal analysis, J.O. and H.X.; Investigation, J.O. and H.X.;

Resources, J.O. and H.X.; data curation, J.O. and K.W.; writing—original draft preparation, J.O.; writing—review and editing, J.O., H.X, and Y.Z.; visualization, J.O.; supervision, H.X.; project administration, J.O. and H.X.; and funding acquisition, H.X.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Lay, D.C.; Fulton, R.M.; Hester, P.Y.; Karcher, D.M.; Kjaer, J.B.; Mench, J.A.; Mullens, B.A.; Newberry, R.C.; Nicol, C.J.; O’Sullivan, N.P.; et al. Hen welfare in different housing systems. Poult. Sci. 2011.

2. Appleby, M.C.; Hughes, B.O. Welfare of laying hens in cages and alternative systems: environmental, physical and behavioural aspects. Worlds. Poult. Sci. J. 1991, 47, 109–128.

3. Tauson, R. Management and housing systems for layers – effects on welfare and production. Proc. Nutr. Soc. 2005, 61, 477–490.

4. Ochs, D.S.; Wolf, C.A.; Widmar, N.J.O.; Bir, C. Consumer perceptions of egg-laying hen housing systems. Poult. Sci. 2018, 97, 3390–3396.

5. Mench, J.A. Farm animal welfare in the U.S.A.: Farming practices, research, education, regulation, and assurance programs. Appl. Anim. Behav. Sci. 2008, 113, 298–312.

6. Chai, L.; Xin, H.; Zhao, Y.; Wang, T.; Soupir, M.; Liu, K. Mitigating Ammonia and PM Generation of Cage-Free Henhouse Litter with Solid Additive and Liquid Spray. Trans. ASABE 2018, 61, 287–294.

7. Cooper, J.J.; Appleby, M.C. Nesting behaviour of hens: Effects of experience on motivation. Appl. Anim. Behav. Sci. 1995, 42, 283–295.

8. Struelens, E.; Van Nuffel, A.; Tuyttens, F.A.M.; Audoorn, L.; Vranken, E.; Zoons, J.; Berckmans, D.; Ödberg, F.; Van Dongen, S.; Sonck, B. Influence of nest seclusion and nesting material on pre-laying behaviour of laying hens. Appl. Anim. Behav. Sci. 2008, 112, 106–119.

9. Struelens, E.; Tuyttens, F.A.M.; Janssen, A.; Leroy, T.; Audoorn, L.; Vranken, E.; De Baere, K.; Ödberg, F.; Berckmans, D.; Zoons, J.; et al. Design of laying nests in furnished cages: influence of nesting material, nest box position and seclusion. Br. Poult. Sci. 2005, 46, 9–15.

10. Zupan, M.; Kruschwitz, A.; Huber-Eicher, B. The influence of light intensity during early exposure to colours on the choice of nest colours by laying hens. Appl. Anim. Behav. Sci. 2007, 105, 154–164.

11. Ringgenberg, N.; Fröhlich, E.K.F.F.; Harlander-Matauschek, A.; Toscano, M.J.; Würbel, H.; Roth, B.A. Effects of variation in nest curtain design on pre-laying behaviour of domestic hens. Appl. Anim. Behav. Sci. 2015, 170, 34–43.

12. Nicol, C.J. Development, direction, and damage limitation: Social learning in domestic fowl. Anim. Learn. Behav. 2004, 32, 72–81.

13. Cooper, J.J.; Appleby, M.C. Individual variation in prelaying behaviour and the incidence of floor eggs. Br. Poult. Sci. 1996, 37, 245–253.

14. Freire, R.; Appleby, M.C.; Hughes, B.O. Assessment of pre-laying motivation in the domestic hen using social interaction. Anim. Behav. 1997, 54, 313–319.

15. Reynard, M.; Savory, C. Oviposition delays induced by social stress are reversed by treatment with the beta-adrenergic blocking agent propranolol. Poult. Sci. 1997, 76, 1315–1317.

16. Appleby, M.C.; Smith, S.F. Design of nest boxes for laying cages. Br. Poult. Sci. 1991, 32, 667–678.

17. Lundberg, A.; Keeling, L.J. The impact of social factors on nesting in laying hens (Gallus gallus domesticus). Appl. Anim. Behav. Sci. 1999, 64, 57–69.

18. Riber, A.B. Development with age of nest box use and gregarious nesting in laying hens. Appl. Anim. Behav. Sci. 2010, 123, 24–31.

19. Appleby, M.C.; Maguire, S.N.; McRae, H.E. Nesting and floor laying by domestic hens in a commercial flock. Br. Poult. Sci. 1986, 27, 75–82.

20. Voulodimos, A.S.; Patrikakis, C.Z.; Sideridis, A.B.; Ntafis, V.A.; Xylouri, E.M. A complete farm management system based on animal identification using RFID technology. Comput. Electron. Agric. 2010, 70, 380–388.

21. Brown-Brandl, T.M.; Eigenberg, R.A. Development of a livestock feeding behavior monitoring system. Am. Soc. Agric. Biol. Eng. 2011, 54, 1913–1920.

22. Maselyne, J.; Saeys, W.; De Ketelaere, B.; Mertens, K.; Vangeyte, J.; Hessel, E.F.; Millet, S.; Nuffel, A. Van Validation of a High Frequency Radio Frequency Identification (HF RFID) system for registering feeding patterns of growing-finishing pigs. Comput. Electron. Agric. 2014, 102, 10–18.

23. Li, G.; Zhao, Y.; Hailey, R.; Zhang, N.; Liang, Y.; Purswell, J.L. An ultra-high frequency radio frequency identification system for studying individual feeding and drinking behaviors of group-housed broilers. animal 2019, 1–10.

24. Nakarmi, A.; Tang, L.; Xin, H. Automated Tracking and Behavior Quantification of Laying Hens Using 3D Computer Vision and Radio Frequency Identification Technologies. Trans. ASABE 2014, 57, 1455–1472.

25. Sales, G.T.; Green, A.R.; Gates, R.S.; Brown-Brandl, T.M.; Eigenberg, R.A. Quantifying detection performance of a passive low-frequency RFID system in an environmental preference chamber for laying hens. Comput. Electron. Agric. 2015, 114, 261–268.

26. Campbell, D.L.M.; Hinch, G.N.; Downing, J.A.; Lee, C. Outdoor stocking density in free-range laying hens: effects on behaviour and welfare. animal 2017, 11, 1036–1045.

27. Siegford, J.; Berezowski, J.; Biswas, S.; Daigle, C.; Gebhardt-Henrich, S.; Hernandez, C.; Thurner, S.; Toscano, M. Assessing Activity and Location of Individual Laying Hens in Large Groups Using Modern Technology. Animals 2016, 6, 10.

28. Li, L.; Zhao, Y.; Oliveira, J.; Verhoijsen, W.; Liu, K.; Xin, H. A UHF RFID System for Studying Individual Feeding and Nesting Behaviors of Group-Housed Laying Hens. Trans. ASABE 2017, 60, 1337–1347.

29. Oliveira, J.L.; Xin, H.; Wu, H. Impact of feeder space on laying hen feeding behavior and production performance in enriched colony housing. Animal 2018, 1–10.

30. Rietveld-Piepers, B.; Blokhuis, H.J.; Wiepkema, P.R. Egg-laying behaviour and nest-site selection of domestic hens kept in small floor-pens. Appl. Anim. Behav. Sci. 1985, 14, 75–88.

31. Reynard, M.; Savory, C.J. Stress-induced oviposition delays in laying hens: Duration and consequences for eggshell quality. Br. Poult. Sci. 1999, 40, 585–591.

32. Shimmura, T.; Eguchi, Y.; Uetake, K.; Tanaka, T. Effects of separation of resources on behaviour of high-, medium- and low-ranked hens in furnished cages. Appl. Anim. Behav. Sci. 2008, 113, 74–86.

33. Stämpfli, K.; Roth, B.A.; Buchwalder, T.; Fröhlich, E.K.F. Influence of nest-floor slope on the nest choice of laying hens. Appl. Anim. Behav. Sci. 2011, 135, 286–292.

34. Wall, H.; Tauson, R.; Elwinger, K. Pop hole passages and welfare in furnished cages for laying hens. Br. Poult. Sci. 2004, 45, 20–27.

35. Cronin, G.M.; Barnett, J.L.; Hemsworth, P.H. The importance of pre-laying behaviour and nest boxes for laying hen welfare: a review. 2012.

36. Tactacan, G.B.; Guenter, W.; Lewis, N.J.; Rodriguez-Lecompte, J.C.; House, J.D. Performance and welfare of laying hens in conventional and enriched cages. Poult. Sci. 2009, 88, 698–707.

37. Buchwalder, T.; Fröhlich, E.K. Assessment of colony nests for laying hens in conjunction with the authorization procedure. Appl. Anim. Behav. Sci. 2011, 134, 64–71.

38. Ringgenberg, N.; Fröhlich, E.K.F.F.; Harlander-Matauschek, A.; Würbel, H.; Roth, B.A. Does nest size matter to laying hens? Appl. Anim. Behav. Sci. 2014, 155, 66–73.

39. Wood-Gush, D.G.M.; Vestergaard, K. Exploratory behavior and the welfare of intensively kept animals. J. Agric. Ethics 1989, 2, 161–169.

40. Villanueva, S.; Ali, A.B.A.; Campbell, D.L.M.; Siegford, J.M. Nest use and patterns of egg laying and damage by 4 strains of laying hens in an aviary system. Poult. Sci. 2017, 96, 3011–3020.

41. Lentfer, T.L.; Gebhardt-Henrich, S.G.; Fröhlich, E.K.F.; von Borell, E. Influence of nest site on the behaviour of laying hens. Appl. Anim. Behav. Sci. 2011, 135, 70–77.

42. Appleby, M.C.; McRae, H.E.; Peitz, B.E. The effect of light on the choice of nests by domestic hens. Appl. Anim. Ethol. 1984, 11, 249–254.

43. Hunniford, M.E.; Torrey, S.; Bédécarrats, G.; Duncan, I.J.H.H.; Widowski, T.M. Evidence of competition for nest sites by laying hens in large furnished cages. Appl. Anim. Behav. Sci. 2014, 161, 95–104.

44. Hunniford, M.E.; Woolcott, C.; Siegford, J.; Widowski, T.M. Nesting behavior of Hy-Line hens in modified enriched colony cages. Poult. Sci. 2017, 96, 1515–1523.

45. Odihambo Mumma, J.; Thaxton, J.P.; Vizzier-Thaxton, Y.; Dodson, W.L. Physiological Stress in Laying Hens. Poult. Sci. 2006, 85, 761–769.

46. Oliveira, J.L.; Xin, H.; Chai, L.; Millman, S.T. Effects of litter floor access and inclusion of experienced hens in aviary housing on floor eggs , litter condition , air quality , and hen welfare. Poult. Sci. 2019.

47. Taylor, T.G. How an Eggshell Is Made. 1970, 222, 88–97.

48. Perrins, C.M. Eggs, egg formation and the timing of breeding. Ibis (Lond. 1859). 1996, 138, 2–15.

49. Wolc, A.; Jankowski, T.; Arango, J.; Settar, P.; Fulton, J.E.; O'Sullivan, N.P.; Dekkers, J.C.M. Investigating the genetic determination of clutch traits in laying hens. Poult. Sci. 2019, 98, 39–45.

50. Samiullah, S.; Roberts, J.; Chousalkar, K. Oviposition time, flock age, and egg position in clutch in relation to brown eggshell color in laying hens. Poult. Sci. 2016, 95, 2052–2057.

51. Hunniford, M.E.; Widowski, T.M. Rearing environment and laying location affect pre-laying behaviour in enriched cages. Appl. Anim. Behav. Sci. 2016, 181, 205–213.

52. Abeyesinghe, S.M.; Drewe, J.A.; Asher, L.; Wathes, C.M.; Collins, L.M. Do hens have friends? Appl. Anim. Behav. Sci. 2013, 143, 61–66.

CHAPTER 5. EFFECTS OF LITTER FLOOR ACCESS AND INCLUSION OF EXPERIENCED HENS IN AVIARY HOUSING ON FLOOR EGGS, LITTER CONDITION, AIR QUALITY, AND HEN WELFARE

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Abstract

With different Cage-free (CF) housing styles and management schemes, retailers have developed their own CF criteria. One highly debated aspect is if hens may be kept inside the system for part of the day – during the first few hours after lights-on. Research is lacking regarding the impacts of such a practice on hen welfare, incidence of eggs laid on the litter floor, litter condition, and air quality. This 14-month field study was conducted to help assess such impacts. Hens (DeKalb White) in an aviary house (50,000-hen nominal capacity) were allowed to have full litter access (FLA) *vs.* part-time litter access (PLA) from 10:50 h to 21:00 h, coupled with the absence or presence of experienced hens (1.5% of the population), hence a 2 × 2 factorial arrangement. The measured variables included: a) incidence of floor eggs, b) percentage of birds remaining on litter floor at night, c) mortality, d) body weight (BW) and BW uniformity, e) litter condition (depth, moisture content, texture, amount removed, and bacteria concentration), f) environmental conditions, and g) welfare conditions (10 variables). Compared to FLA, PLA had a significantly lower incidence of floor eggs (1.4 ± 0.1 *vs.* 12.6 ± 1.1 eggs per hen housed as of 76 weeks of age (WOA), i.e., ~89% reduction), less manure deposition on the floor (0.53 ± 0.02 *vs.* 1.05 ± 0.04 kg/100 hens/d, dry basis, i.e., ~50% reduction), and lower ammonia concentrations due to drier litter (averaging 22% lower).

Inclusion of 1.5% experienced hens in the young flock did not show benefit of reducing the incidence of floor eggs (P = 0.48). The percentage of hens remaining on the floor at night was low (< 0.01%) in all cases from 24 WOA onward. No differences were detected between FLA and PLA in hen welfare conditions, mortality, BW, BW uniformity, bacteria concentration in the litter, air temperature or relative humidity.

Keywords: food safety, poultry management, animal welfare, air quality

Introduction

Cage-free (CF) egg production has been a topic of increasing importance in the United States due to pledges made by food retailers and restaurants to source only CF eggs by certain year (e.g., 2025) (Chai et al., 2018a). However, issues or challenges associated with CF production remain to be addressed with regards to food quality and safety (Holt et al., 2011; Hannah et al., 2011; Jones et al., 2015), litter usage and dust bathing motivation (Colson et al., 2007; Campbell et al., 2016a; Ali et al., 2016), indoor air quality and emissions (Zhao et al., 2013, 2015, 2016), and welfare (Widowski et al., 2016; Blatchford et al., 2016; Louton et al., 2017). With different CF styles and management schemes, retailers have developed their own CF criteria (Mench et al., 2011; Scott et al., 2017). One of the debated criteria is concerning litter access, namely, whether the laying hens should be provided full litter access throughout the day to be qualified as CF egg production, as compared to temporarily confining the hens in the aviary system (one type of CF system) during oviposition period in early morning. Research is lacking regarding the impact of such practice on hens' welfare, floor eggs, litter and environmental conditions.

One of the main improvements of CF systems is the inclusion of litter floor area, allowing the hens to express their natural behavior of dust bathing (Colson et al., 2007). In previous studies, laying hens performed dust bathing throughout the day, but the activity peaked in late morning and afternoon (Vestergaard, 1982; Hansen, 1994; Campbell et al., 2016a). In the morning, the hens' priority is the pre-laying and laying motivation (Hunniford et al., 2014, 2017); and depending on where the eggs are nested the hens can be classified as nest-layers or floor-layers (Sherwin and Nicol, 1993; Cooper and Appleby, 1996; Zupan et al., 2008; Kruschwitz et al., 2008). Oviposition place is one of the biggest concerns in CF systems because floor eggs are directly linked to food safety and economic issues (De Reu et al., 2008; Jones et al., 2015). Therefore, it is essential to develop and test strategies to reduce the incidence of floor eggs in commercial CF systems.

Previous studies have demonstrated that animals exposed to the behavior of a trained demonstrator subsequently acquire the relevant novel response more rapidly; however social factors have an important influence in determining whether social learning will occur (Nicol and Pope, 1992, 1994). From this perspective, it may be worthy of investigating if hens experienced in laying eggs in the colony nest in aviary systems can help training or motivating the novice young hens to use the colony nests.

The inherent feature of litter area access in CF production systems is that part of the hen manure is deposited on the floor and remain on it for an extended period, as compared to 100% of the manure deposited onto the manure belt underneath each cage tier and frequently removed from houses. The end result is a much less desirable indoor air quality for both the animals and the caretakers and elevated air emissions (Jones et al., 2015; Zhao et al., 2015; Chai et al., 2018a). Then it is reasonable to expect that managing litter access by the hens or the amount of manure deposition on the litter floor can be conducive to improving the litter conditions and indoor air quality. On the other hand, there is a general concern that limiting litter access of the hens would affect expression of animal's natural behaviors, which may lead to compromised welfare (Alm et al., 2015). There has also been anecdotal claim that confining hens inside the systems negatively affect flock uniformity. However, data are lacking to substantiate the concerns or claims.

Therefore, the objective of this study was to evaluate the effects of full litter access (**FLA**) *vs.* part-time litter access (**PLA**) and inclusion of experienced hens or not on occurrence of floor eggs, litter condition, indoor air quality, and hen welfare through a long-term field study involving a commercial aviary hen housing system. The hypotheses were that a) PLA would be beneficial in reducing floor eggs, improving litter condition on the floor and thus ammonia generation while not adversely affecting mortality, body weight (BW) and BW uniformity, or welfare of the hens; and b) inclusion of 1.5% experienced birds would be conducive to training a young flock of hens in their nesting behavior, hence reducing incidence of floor eggs.

Materials and methods

Animals and housing

An aviary CF henhouse (153m L x 21m W x 3m H) with concrete floor and containing Big Dutchman Natura 60™ aviary system was used in this field study, initially housing 51,405 DeKalb White pullets at 17 weeks of age (**WOA**). The pullets had been beak trimmed at hatchery and reared in an aviary pullet house.

The aviary house featured system doors that could be controlled to stay open or closed. The henhouse was comprised of 40 sections, of which 32 were enrolled in the four experimental treatments (eight sections per treatment). Sixteen (16) outer sections (15m L x 3m W x 3m H) were located adjacent to the sidewalls of the house, each containing 857 birds.

16 inner sections (15m L x 6m W x 3m H) were located in the interior of the house, each containing 1,714 birds. Hence, approximately 10,280 pullets were allocated to each treatment balanced over four inner sections and four outer sections. The stocking density was 525 cm^2 hen^{-1} on the litter floor and 620 cm^2 hen^{-1} in the aviary system.

The four experimental treatments were: 1) FLA with pullets only (i.e. absence of experienced hens) (**FLA_P**), 2) FLA with pullets plus 1.5% experienced hens (**FLA_E**), 3) PLA (10:50h - 21:00h per day) with pullets only (**PLA_P**), and 4) PLA with pullets plus 1.5% experienced hens (**PLA_E**). The lighting program ranged from 12 to 16 hr depending on the hens' age. After 24 WOA, the light came on at 05:00h and started going off at 21:00h, with a 45-min dimming period. In this paper, the word "regimen(s)" is used when comparing the effect of litter access (PLA *vs.* FLA) while the word "treatment(s)" is used when evaluating the effect of litter access nested with the inclusion of experienced hens or not (FLA_P, FLA_E, PLA_P and PLA_E).

Upon transfer to the laying house, all pullets were kept inside the system for 10 d to ensure familiarity with the system (e.g., location of feed, water, and the colony nest) before starting the respective treatments. The entire floor area of the house was initially covered with approximately 340 kg of wood shavings, uniformly distributed with 7 kg in each of the outer sections and 14 kg in each of the inner sections, before the litter access was provided. After day 10, the system doors in the FLA regimen were opened and remained open, and experienced hens (1.5% population) were introduced to the FLA_E treatment. Hens in the PLA regimen followed the management practice of being kept inside the system for a total of four weeks to ensure they would start laying eggs in the colony nest before having access to the litter floor area. At 22 WOA the PLA birds were allowed daily access to the litter floor from 10:50h (after the general oviposition time) until lights-off (21:00h), and experienced hens (1.5% population) were introduced to the PLA_E treatment. For convenience and biosecurity, experienced hens were obtained from another aviary house on the same farm; experience hens were Bovan Whites at 49 and 53 WOA when enrolled in the FLA_E and PLA_E treatments, respectively. Feed was provided to the hens, via feed conveyor chains, four times a day at 05:30h, 09:30h, 14:30h, and 16:30h.

Measurements and data collection

Floor eggs

The number of floor eggs were counted manually, once a day, and recorded in the checklist provided in each of the 32 sections.

Birds on the floor at night

The birds remaining on the litter floor after the lights off at night were counted manually, early in the morning (before lights came on), and recorded in the checklist in each section. The measurement was taken daily during the first 3 months, and weekly afterwards.

Mortality

The number of dead birds in each section was counted manually and recorded on the checklist once a day.

BW and BW uniformity

Fifty birds per treatment were randomly selected and weighed weekly. Average and standard error were calculated for each of the four treatments.

Litter conditions

Litter samples were collected from the litter floor once a month, in three different locations (under the system, under the outside perch at the litter area, and in the open litter area) of 16 sections in the henhouse (4 sections per treatment). Litter texture was visually observed once a month in all 32 sections. Litter depth was measured at three locations in each of the 32 sections (under the system, under the outside perch at the litter area, and in the open litter area) using a wooden stick and a metal ruler. The wooden stick was inserted into the litter until reaching the concrete floor. A line was drawn on the stick at the litter surface level and the depth measured with the metal ruler. Litter moisture content was determined by oven drying of 10 g litter sample at 105 °C for 24 h. The texture of litter area in each section was classified as “loose”, “partially caked” (presence of caking in < 50% of the litter area), and “caked” (presence of caking in > 50% of the litter area).

Litter on the floor in both regimens was removed from the aviary house during weeks 37/38, 54/55, and 77/78 due to excessive accumulation in the FLA regimen, and the amount of litter removed (volume and weight) was determined and recorded. Litter samples were tested for bacteria concentration at the end of the experiment. A composite litter sample (approximately 50 g) representing three locations of each section (under the system, under the outside perch at the litter area, and in the open litter area) was collected by scooping the litter; and this was done for 16 sections (i.e., 4 sections per treatment). The 16 collected samples were transported in an ice-chest cooler to the analytical laboratory at Iowa State University, where one gram of litter (as is) from each sample was transferred into 9 mL physiological saline solution, and homogenized by vortexing for 30 s and serially diluted (1:8). Viable counts of total bacteria were determined by plating 0.1 mL portions onto plates of trypticase soy agar (TSA) (Catalog No. R455002, Fisher Scientific, Hanover Park, IL).

The plates were aerobically incubated at 37°C for 24 h. The colonies formed on plates (30 to 300 colonies) were counted and used for calculating bacteria concentration by the following equation (Eq. 1) (Zhao et al., 2016; Chai et al., 2018b):

$$BC = log_{10}\left(\frac{n \times 10^{d}}{V_p} \times V_s\right) \quad (1)$$

where BC is the bacteria concentration (logCFU/g), CFU is colony forming unit; *n* is the number of colonies found in the plate (30 to 300 colonies), V_p is the volume of portion plated (mL), *d* is the serial dilution factor (0 for undiluted sample and 1 for 10-fold diluted sample, etc.), and V_s is the total volume of original liquid sample (mL).

Environmental conditions

Ammonia (NH_3) concentration (ppm) was measured at the litter perch level by using three different instruments: 1) detection tubes used with a hand pump (RAE Systems®, Sunnyvale, CA), 2) electrochemical NH_3 detector (Honeywell®, Sunnyvale, CA), and 3) electrochemical NH_3 detector (MSA Altair®, Mine, WV). The detection tubes were used in 8 sections (2 per treatment), while the electrochemical detectors were used in all 32 sections. Air temperature, relative humidity (RH), and carbon dioxide (CO_2) concentrations were continuously monitored and recorded at 10-min intervals with 11 portable T/RH data loggers (Hobo® MX2301, Onset, Bourne, MA) and two T/RH/CO_2 loggers (Hobo® MX1102, Onset, Bourne, MA). The loggers were placed inside the system (6), in the litter area (6), and outside the aviary house (1).

Welfare status

Welfare assessment of the hens was performed at 72 WOA, with 200 hens randomly selected from 20 sections (5 sections per treatment, 10 hens per section). Methodology for the welfare assessment was adapted from the procedures of Welfare Quality ® Assessment Protocol for Poultry (Welfare Quality®, 2009). The assessment method was applied to individual hens rather than at the flock level. Two professionals who were versed in poultry welfare assessment and blind to treatment allocations simultaneously evaluated five hens in each of the 20 sections of the house (5 hens x 20 sections = 100 hens/assessor x 2 assessors = 200 hens). Within each section, research personnel randomly selected five hens from the litter area and five hens from inside the aviary system to present to the assessors for evaluation; number of hens that each assessor evaluated from litter and aviary locations was balanced over the 20 sections.

Each hen was evaluated according to the following welfare characteristics: **Plumage damage** was scored at a maximum of 14 points (sum of the scores from the plumage of head, neck, back, rump, crop, keel and belly). Each area was scored as 0 (no or slight wear), 1 (moderate wear) or 2 (at least one featherless area ≥ 5 cm in diameter). **Cleanliness** was scored at a maximum of 3 points according to the perceived area of manure soiling on breast, back, rump, belly and wings, namely, 0 (no manure soiling), 1 (slight manure soiling), 2 (moderate manure soiling), or 3 (mostly dirty). **Keel bone deformation** was evaluated via palpation to detect abnormal curvature and scored as 0 if no deformation or 2 otherwise. **Comb pecking wounds** was scored at a maximum of 3 points, i.e., 0 (no evidence of pecking wounds), 1 (1 or 2 pecking wounds), or 2 (3 or more pecking wounds). Comb was also evaluated to investigate signs of **comb abnormality** (blue or black areas, very pale combs, or dried areas). The hens were scored as 0 if no evidence of comb abnormality or 1 otherwise. Foot health was assessed to evaluate **foot pad dermatitis**. The hens were scored at a maximum of 2 points, i.e., 0 (intact feet), 1 (minimal lesion on foot pad), or 2 (visible inflammation, swollen foot). **Toe damage** was also evaluated to investigate signs of wounds or missing parts, i.e., 0 (no toe damage) or 1 otherwise. **Claw length** was scored as 0 if short (< 1 cm) or 1 long (≥ 1cm). **Lesions in the skin** (wounds not healed) was evaluated and scored as 0 (no lesion), 1 (lesion < 2 cm in diameter), or 2 (lesion ≥ 2 cm in diameter). All hens in this study were beak trimmed, therefore the condition of **beak trimming** was evaluated and scored as 1 (light to moderate trimming with moderate to no abnormalities) or 2 (severe trimming and clear abnormalities

The protocols were approved by the Iowa State University Institutional Animal Care and Use Committee (IACUC).

Statistical analyses

The sections were considered as the experimental units. Data were tested for homoscedasticity and normality, and transformed when necessary. Mixed model analysis of variance and logistic regression were used to evaluate the effects of litter access management and inclusion of 1.5% experienced hens on the amount of floor eggs, hens on the litter floor at night, mortality, BW, BW uniformity, and welfare status.

The effect of litter access management was further investigated on litter depth, amount of litter on the floor (removed), and litter moisture content and litter bacteria concentration (all using the mixed model), as well as litter texture (using logistic regression model). Litter conditions could in turn affect ammonia generation and thus concentration.

Air temperature, RH and CO_2 concentration were evaluated to investigate changes in the microenvironment (inside the system *vs.* litter area) and in the period of the day (light vs. dark) to observe the homogeneity or heterogeneity inside the aviary housing. The effect of litter access management on temperature and RH were also investigated.

Statistical analysis was performed using JMP 13.2.1 (SAS Campus Drive, Cary, NC). When appropriate, repeated measures analysis was incorporated into the mixed model, and regression analysis was performed. A p-value of 0.05 or less indicates a significant difference among the treatments. Unless otherwise specified, data are presented as least squares means along with the standard error of the mean.

Results

Floor eggs

Weekly percentage of floor eggs was not affected by inclusion of 1.5% experienced hens in the young flock ($P = 0.48$), but it was affected by the litter access management ($P < 0.01$). No effect of interaction between inclusion of 1.5% experienced hens and litter access management was found ($P = 0.54$). Overall mean weekly percentage of floor eggs was 4.15 ± 1.53% in FLA and 0.29 ± 0.11% in PLA; 1.05 ± 0.39% with inclusion of 1.5% experienced hens and 1.12 ± 0.42% without. The cumulative floor eggs per 1,000 hens housed as of 76 WOA was 12,625 ± 1,111 and 1,374 ± 148 (i.e., 12.6 ± 1.1 and 1.4 ± 0.1 eggs per hen housed) for the FLA and PLA regimen, respectively ($P < 0.001$). The percentages of weekly and cumulative floor eggs per treatment (mean and SE) are presented in Figure 5.1. The percentage of floor eggs was higher in the first two weeks in FLA (> 40%), and reduced gradually until week 25. After the PLA birds had daily litter access (22 WOA), the difference in percentage of floor eggs became evident between the FLA and PLA regimens.

The litter floor cleaning was performed three times when hens were 37/38, 54/55 and 77/78 WOA, during which the system was kept closed for all treatments. The abrupt reduction in percentage of floor eggs following the system closure continued after the doors were reopened, with a trend of increase in the subsequent week. Although there was no statistical effect of including experienced hens, FLA_P showed a numerically higher percentage of floor eggs than FLA_E after the first cleaning period (39 WOA). The overall percentage of floor eggs was 3.33 ± 0.48% in FLA_P and 2.74 ± 0.40% in FLA_E ($P = 0.77$). The non-significant effect of including the experienced hens was also shown in the PLA regimen, with overall percentage of floor eggs being 0.26 ± 0.04% in PLA_P and 0.26 ± 0.04% in PLA_E ($P = 0.99$).

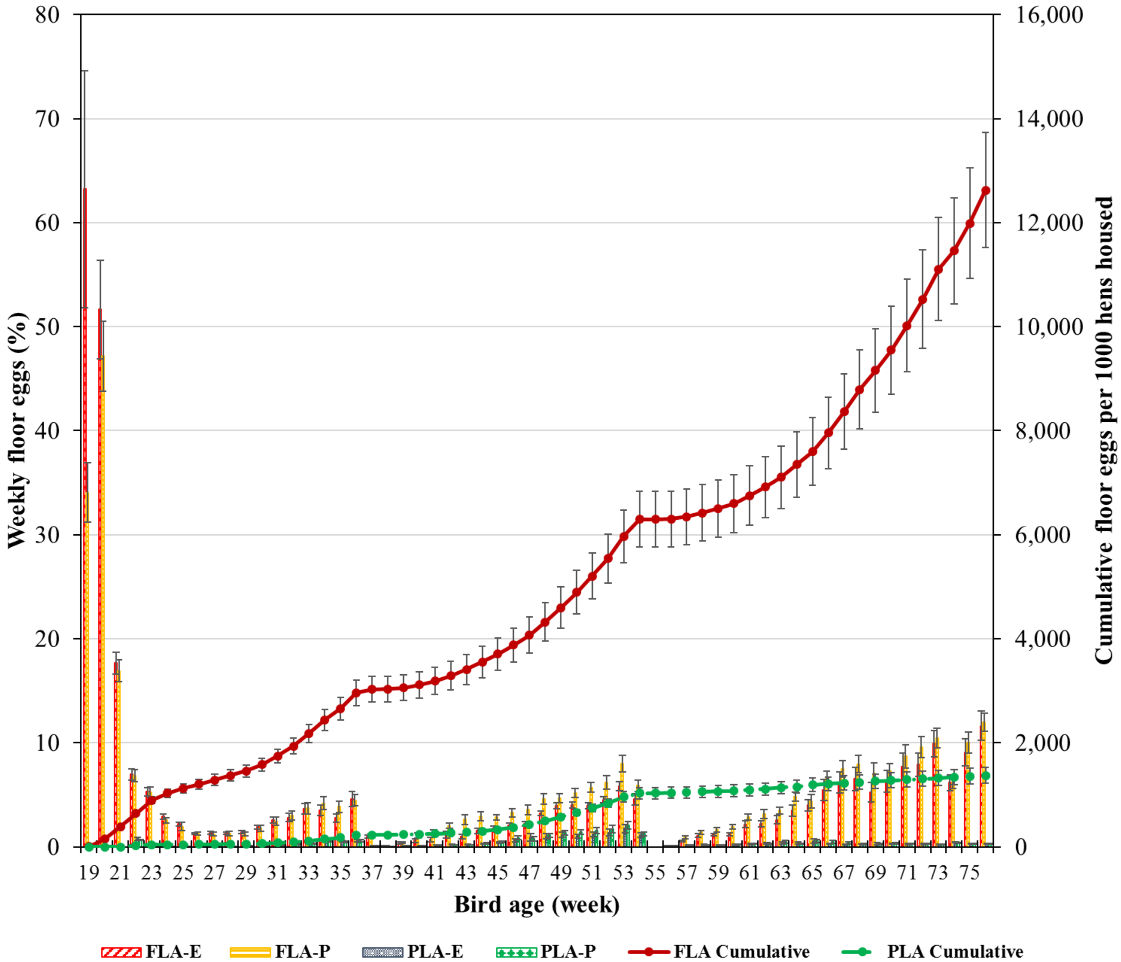

Figure 5.1 *Weekly percentage of floor eggs (bars, %, mean ± SE) and cumulative floor eggs per 1,000 hens housed (lines, %, mean ± SE) of full litter access (FLA) or part-time litter access (PLA) vs. hen age. Hens were kept inside the system during litter removal at 37/38, 54/55 and 77/78 WOA, respectively*.

Hens remaining on litter floor at night

The proportion of birds that stayed outside the system at night was lower than 0.1% and similar for all treatments from 25 WOA onward. Using experienced hens did not affect percentage of hens remaining outside the system at night (P = 0.71). However, the proportion of hens outside the system at night was statistically different between the FLA (0.040 ±

0.002%) and PLA (0.010 ± 0.001%) regimens (P < 0.001). No significant effect of interaction between inclusion of 1.5% experienced hens and litter access management was found (P = 0.85).

Mortality rate

The weekly and cumulative mortality rates for each treatment are presented in Figure 5.2. No effect of litter access or experienced hens on mortality rate was observed. Overall weekly mortality rate was 0.22 ± 0.03% in FLA and 0.21 ± 0.03% in PLA (P = 0.76), and 0.23 ± 0.03% when including 1.5% experienced hens and 0.21 ± 0.03% when not (P = 0.29). No significant effect of interaction between inclusion of 1.5% experienced hens and litter access management was found (P = 0.92).

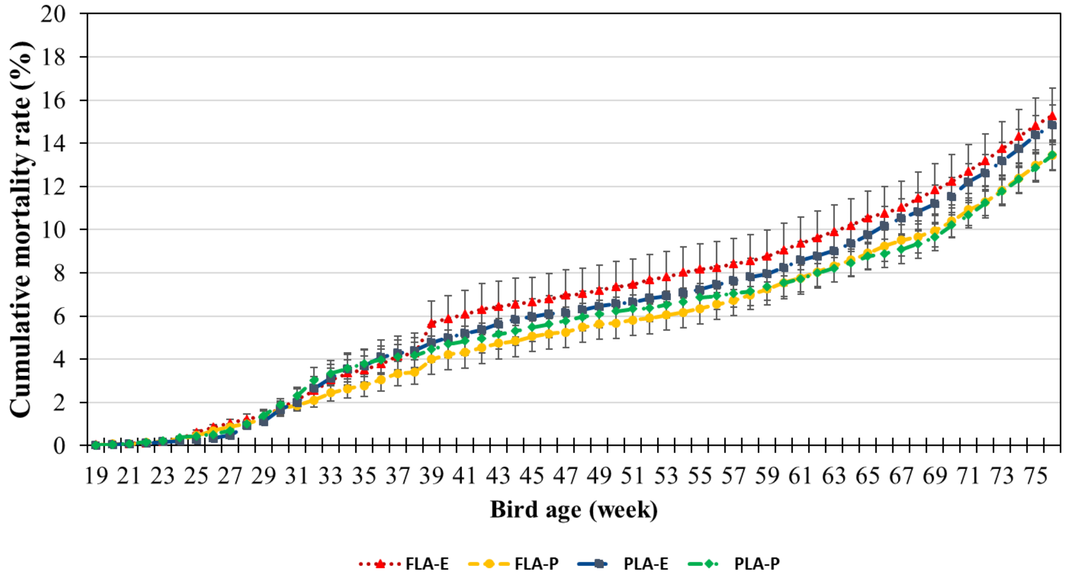

Figure 5.2 *Cumulative mortality (%, mean ± SE) vs. hen age in the four different treatments: 1) Part-time litter access plus 1.5% experienced hens (PLA$_E$), 2) Part-time litter access without experienced hens (PLA$_P$), 3) Full litter access plus 1.5% experienced hens (FLA$_E$), and 4) Full litter access without experienced hens (FLA$_P$).*

The first litter floor cleaning was performed when hens were 37–38 WOA, when the system was kept closed for all regimens. An abrupt increase in mortality rate was observed after the doors were reopened, especially in the FLA_E treatment. Due to the difficulty of locating dead birds during the period of cleaning (between 37 and 38 WOA) and a concurrent shortage of caretakers in the same period, it was possible that some of the mortalities incurred during this period were accounted for in the immediate subsequent week record (39 WOA).

BW and BW uniformity

BW was not affected by litter access management (1.53 ± 0.01 kg in FLA and 1.51 ± 0.01 kg in PLA, $P = 0.30$) or inclusion of experienced hens (1.52 ± 0.01 kg with experienced hens and 1.53 ± 0.01 kg without, $P = 0.87$). Similarly, BW uniformity was not affected by the litter access management (81.5 ± 0.83% in FLA and 82.9 ± 0.83% in PLA, $P = 0.17$) or inclusion of experienced hens (82.4 ± 0.83% with experienced hens and 82.0 ± 0.83% without, $P = 0.81$). No significant effect of interaction between inclusion of 1.5% experienced hens and litter access management was found for BW ($P = 0.61$) or BW uniformity ($P = 0.23$).

Litter conditions

Moisture content of the litter was affected by litter access management (31.3 ± 1.6% in FLA and 20.3 ± 1.1% in PLA, $P < 0.001$). Similarly, litter depth was influenced by access to litter area (3.77 ± 0.09 cm in FLA and 1.64 ± 0.04 cm in PLA, $P < 0.001$). The parameters of the litter conditions are summarized in Figure 5.3.

Litter access management affected the amount of floor litter removed, namely, 1.56 ± 0.06 kg/100 hens/d in FLA and 0.67 ± 0.03 kg/100 hens/d in PLA, as-is basis; or 1.05 ± 0.04 kg/100 hens/d in FLA and 0.53 ± 0.02 kg/100 hens/d in PLA, dry basis ($P < 0.001$). However, no effect of the litter access management was observed in the litter bacteria level, 8.98 ± 0.06 logCFU/g in FLA and 8.92 ± 0.06 logCFU/g in PLA ($P = 0.51$).

Litter texture was significantly different between the FLA and PLA regimens ($P < 0.001$). Overall, FLA litter had 33.1% of area in "caked", 32.5% in "partially caked", and 34.4% in "loose" category. In comparison, the respective proportions of the PLA litter were 0% "caked", 18.8% "partially caked", and 81.3% "loose".

Environmental conditions

Management of litter access had no effect on indoor air temperature (21.7 ± 0.2 ˚C in FLA and 21.7 ± 0.2 ˚C in PLA, $P = 0.91$) or RH (65 ± 1% in FLA and 67 ± 1% in PLA, $P = 0.34$). The location of the hens (system or litter area) did not affect air temperature (21.4 ± 0.2 ˚C in litter and 21.7 ± 0.2 ˚C in system, $P = 0.20$), or RH (66 ± 1% in litter area and 66 ±1% inside the system, $P = 0.53$), but the period of day (light or dark) did affect both temperature (22.6 ± 0.2 ˚C during light period and 20.5 ± 0.2 ˚C during dark period, $P < 0.01$) and RH (64 ± 1% during light period and 67 ± 1% during dark period, $P < 0.001$). There was no statistical evidence of interaction between location (system or litter area) and period of day (light or dark) for temperature ($P = 0.09$) or RH ($P = 0.15$).

The CO_2 concentration was 2372 ± 345 ppm in litter area and 2034 ± 345 ppm inside the system ($P = 0.37$), and no significant differences were found between light and dark period ($P = 0.89$) or in the interaction between location and period of day ($P = 0.94$). Air temperature, RH and CO_2 profiles are shown in Figure 5.4.

The relationship between indoor CO_2 concentration (ppm) and ambient temperature (˚C), valid for the temperature range -10 ˚C < t < 26 ˚C, can be described by the following empirical model (Eq. 2):

$$CO_2 = 2.504\, t^2 - 131.8\, t + 2577 \; (r^2 = 0.89) \qquad (2)$$

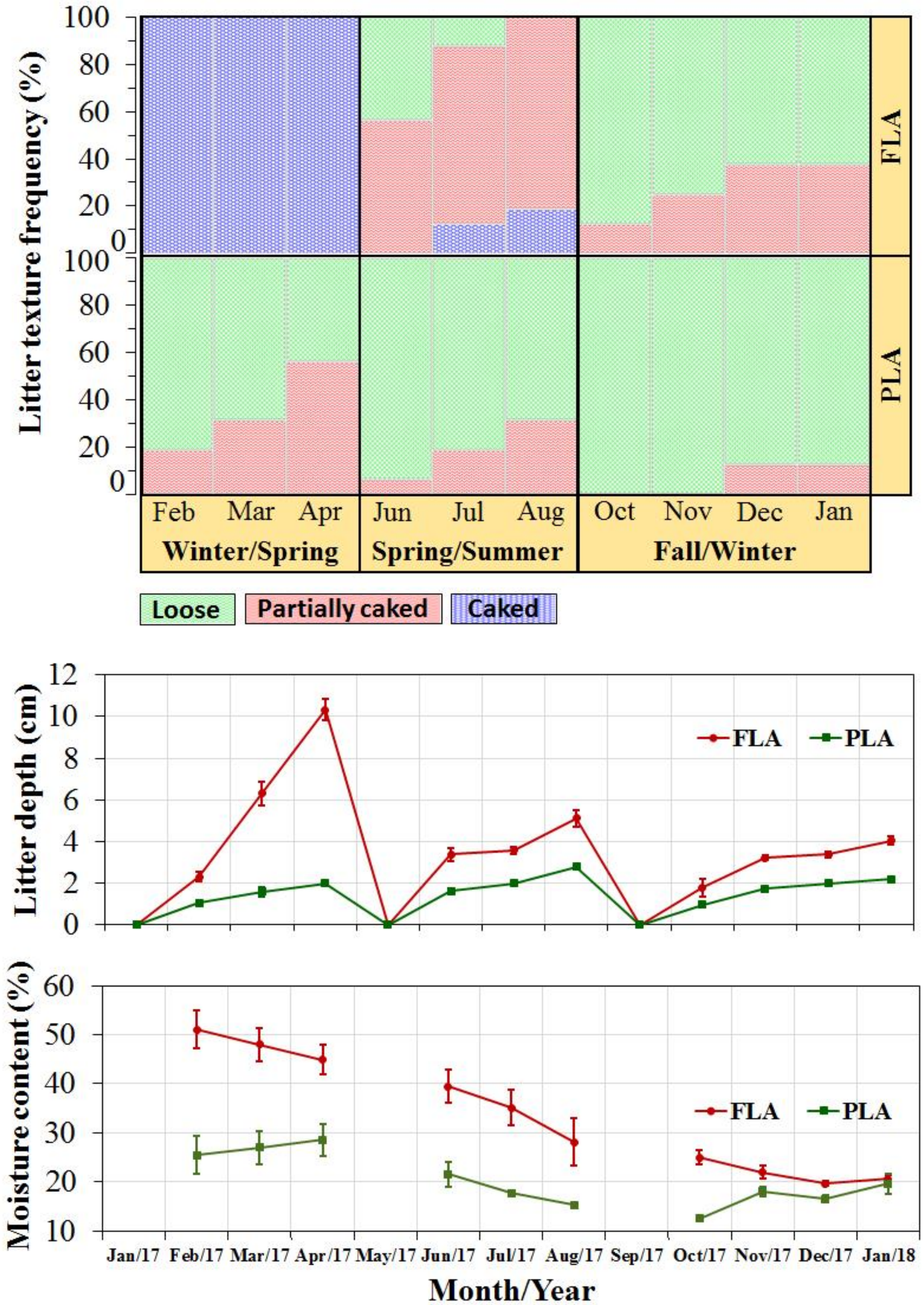

Figure 5.3 *Litter texture (loose, partially caked or caked), depth (cm, mean ± SE) and moisture content (%, mean ± SE) for the full litter access (FLA) and part-time litter access (PLA) regimens during the experimental period (Litter floor was cleaned three times: May/17, September/17 and February/18).*

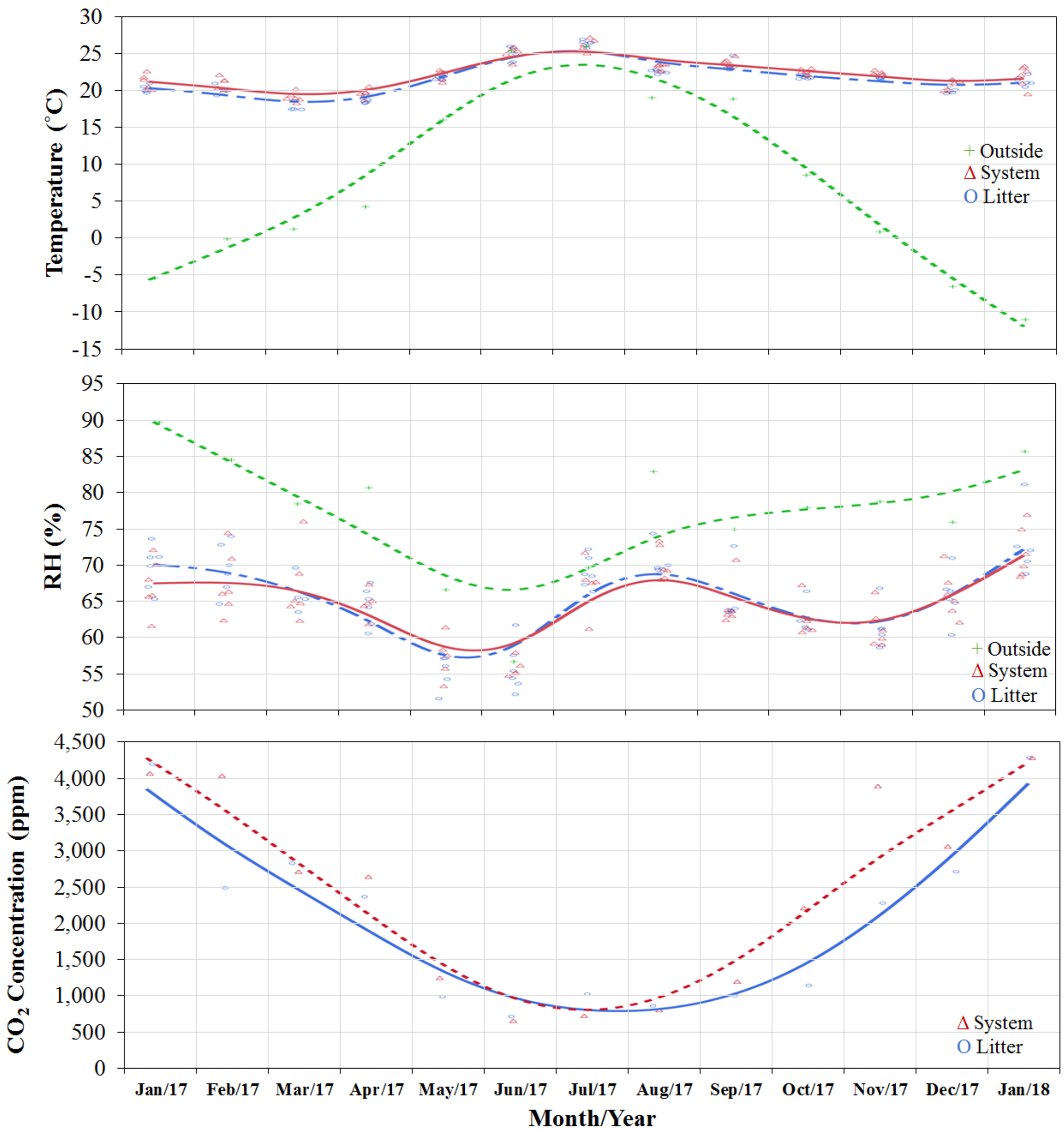

Figure 5.4 *Profiles of air temperature, relative humidity (RH) and CO_2 concentration of the litter area, inside the system, and outside ambient over the experiment period.*

Ammonia concentration was affected by litter access management (17.2 ± 0.8 ppm in FLA and 13.5 ± 0.6 ppm in PLA, $P < 0.001$). Figure 5.5 shows the seasonal profiles of ammonia concentrations in both regimens.

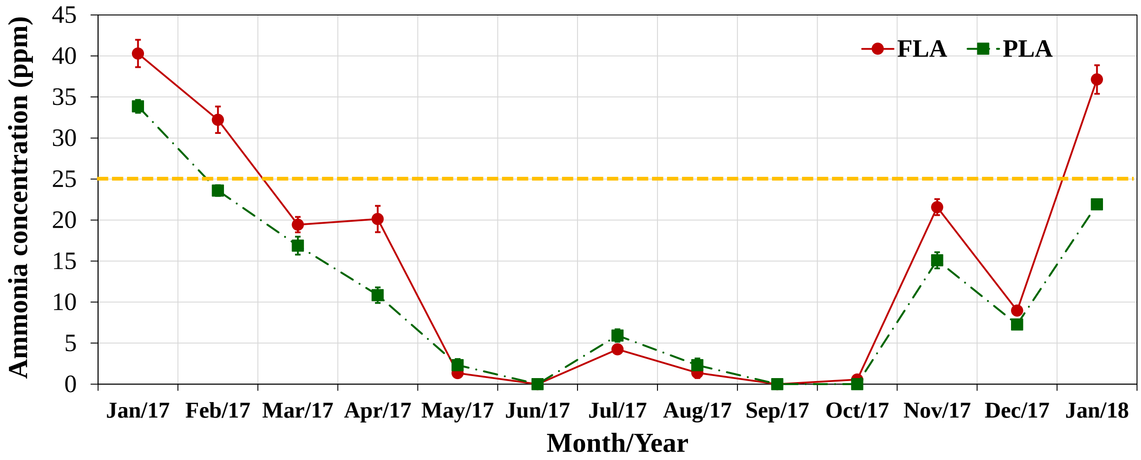

Figure 5.5 *Ammonia concentration (mean ± SE) over the experiment period for the full litter access (FLA) and part-time litter access (PLA) regimens. The horizontal dashed line represents the 8-hr exposure threshold for workers recommended by the National Institute for Occupational Safety and Health (NIOSH).*

Ammonia concentration decreased almost linearly from January 2017 (cold weather) to May 2017 (mild weather). The highest ammonia levels mostly occurred in the FLA sections, presumably arising from the more manure accumulation and higher moisture content of the litter. After March 2017, when ventilation rate increased in response to the warmer weather, ammonia concentrations fell below 25 ppm, the 8-hr exposure threshold for workers recommended by the National Institute for Occupational Safety and Health (NIOSH) as well as the recommended threshold for poultry housing (NIOSH, 2010).

Welfare conditions

During the welfare assessment, panting, piling, enlarged crop, eye pathology, nares discharge or inflammation, enteritis or external parasites were not observed. Data for plumage condition, cleanliness, keel bone deformation, comb pecking, comb abnormality, foot pad dermatitis, claw length, skin lesions, beak trimming and toe damage are presented in Table 5.1.

Table 5.1 *Mean scores and standard error for the welfare status under the different litter access management and inclusion of experienced hens or not: plumage condition, cleanliness, keel bone deformation, comb peck wounds, comb abnormality, foot pad dermatitis, claw length, skin lesion, beak trimming and toe damage*

Welfare variable	Litter Access (LA)			Experienced Hens(EH)			*P-Value*		
	Full	Partial	SE	Yes	No	SE	LA	EH	LA x EH
Plumage condition[1]	4.71	4.97	0.24	4.78	4.90	0.24	0.51	0.62	0.31
Cleanliness[2]	0.43	0.34	0.05	0.35	0.42	0.05	0.33	0.38	0.14
Keel deformation[3]	1.26	1.04	0.10	1.08	1.22	0.10	0.11	0.31	0.64
Comb peck wounds[4]	0.09	0.05	0.03	0.09	0.05	0.03	0.28	0.28	0.54
Comb abnormality[5]	0.01	0.01	0.01	0.01	0.01	0.01	1.00	1.00	0.09
Foot pad dermatitis[6]	0.29	0.38	0.05	0.29	0.38	0.05	0.20	0.20	0.33
Claw length[7]	0.82	0.83	0.04	0.89	0.76	0.04	0.98	**0.01**	0.59
Skin lesions[8]	0.04	0.07	0.02	0.05	0.06	0.02	0.28	0.55	0.28
Beak trimming[9]	1.19	1.18	0.04	1.18	1.19	0.04	0.88	0.88	0.10
Toe damage[10]	0.01	0.02	0.01	0.01	0.01	0.01	0.09	1.00	0.09

[1]Sum of scores from the plumage conditions of head, neck, back, rump, crop, keel and belly. Each area has a score of 0, 1 or 2 (no wear to moderate and featherless) with a maximum overall score of 14.

[2] Score is 0, 1, 2 or 3 as dirtiness increases.

[3] Score is 0 or 2 for intact or deformed keel bone.

[4] Score is 0, 1, 2 or 3 with increasing evidence of pecking wounds.

[5] Score is 0 or 1 for presence or absence of abnormality.

[6] Score is 0, 1 or 2 with increasing evidence of foot pad dermatitis.

[7] Score is 0 or 1 for short or long claws.

[8] Score is 0, 1 or 2 with increasing evidence of lesions in the skin.

[9] Score is 1 or 2 for moderate or severe trimming.

[10] Score is 0 or 1 for presence of absence of toe damage.

The welfare status was not affected by the litter access management or inclusion of 1.5% experienced hens, with the exception of the claw length condition. Hens with long claws were observed more often in the sections without the experienced hens (P = 0.01).

Claw length, keel bone deformity and foot pad dermatitis were the welfare variables with the highest occurrences of the worst conditions. Figure 5.6 shows the frequency of occurrence of each welfare score per treatment. The green color represents the best welfare status where the red color represents the worst.

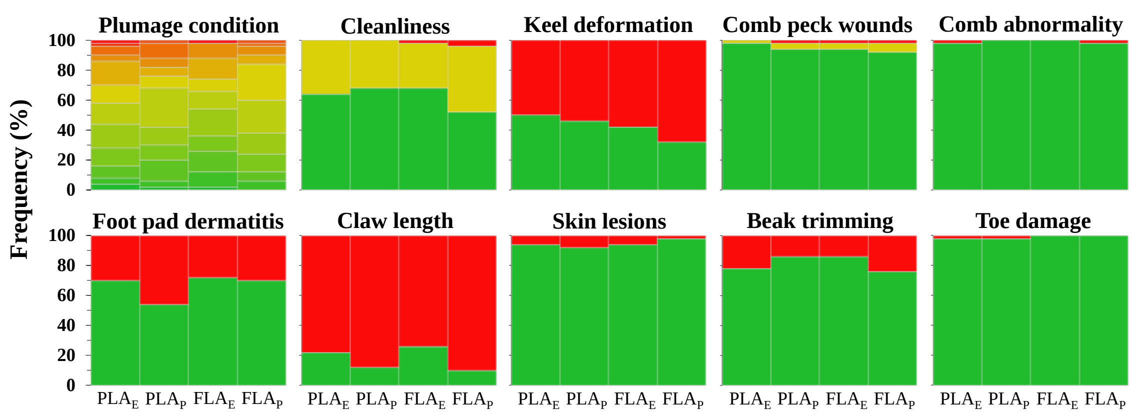

Figure 5.6 *Welfare status of the laying hens in the four different treatments: 1) Part-time litter access plus 1.5% experienced hens (PLAE), 2) Part-time litter access without experienced hens (PLAP), 3) Full litter access plus 1.5% experienced hens (FLAE), and 4) Full litter access without experienced hens (FLAP). The graphs represent the distribution of each welfare aspect, with the color varying from green to red corresponding to best to worst condition respectively.*

Discussion

In the present study, we performed a comprehensive and novel evaluation of the effect of managing litter floor access and including 1.5% of experienced hens on several production traits, welfare status, litter conditions, air quality and thermal conditions. The uniqueness of the study lies in its comprehensive and longer-term (entire flock production cycle) nature of the comparative monitoring under field production conditions.

Floor eggs

The amount of floor eggs decreased with time in the first 8 weeks for all treatments. This trend could be a result of transitioning to stabilization as the birds became more accustomed to using the system structure. Similar result was observed by Cooper and Appleby (1996) who evaluated the incidence of floor eggs from individual laying hens from 22 to 28 WOA and found a reduction from 25% to 5% of eggs laid on the floor, respectively. However, the short period of the Cooper and Appleby study did not allow evaluation of stability of this behavior over the production cycle.

After 28 WOA, we observed a consistent increase in the number of eggs laid on the litter floor for all treatments until the time of floor cleaning when the system was closed for a period of 10 d (Figure 5.1). During this period, the hens were re-trained to use the system (colony nest); and upon allowing for litter area access the percentage of floor eggs was < 1%. In an extensive review on cognition, emotion and behavior of domestic chickens, Marino (2017) reinforced that learning, particularly in a social context, is an important driver of chicken cognition. But information is scarce about how cognitive abilities play out developmentally into maturity in chickens.

The percentage of eggs laid on the floor increased linearly in the subsequent weeks after the access to litter floor area was re-established. It indicates that the hens' preference of laying eggs on the floor over the colony nest was not eliminated by the time that they were locked into the system during the cleaning period. Although we could not assure that the floor eggs were laid by the same hens, some studies showed that floor and nest eggs were consistently laid by the same floor or nest laying hens (Sherwin and Nicol, 1993; Cooper and Appleby, 1996; Zupan et al., 2008; Kruschwitz et al., 2008). Still, the factors that motivate the floor-nesting behavior are unclear: genetic differences (McGibbon, 1976), social dominance (Sherwin and Nicol, 1993), or simply because they do not want to use colony nest (Zupan et al., 2008).

Including 1.5% experienced hens did not impact incidence of floor eggs. However, caution should be taken when drawing conclusions on the effectiveness of including experienced hens because we did not evaluate any inclusion rate other than 1.5%. This result suggests that using experienced hens may not be an effective mean to stimulate the motivation of young hens for using colony nests in aviary systems. Cooper and Appleby (1995) evaluated whether laying hens from conventional cages, deprived of resources that they had never experienced, would present similar level of motivation to use littered nests as those with prior experience, and found that the nest-seeking behavior was independent of prior experience of nesting cues. In an experiment to evaluate nesting behavior and gregarious nesting, Riber (2010) suggested that young inexperienced hens visited the same colony nest as frequently as the experienced hens. In addition, as the young hens gained experience they tended to rely more on their own experience in selection of nest. Richard-Yris and Leboucher (1987) evaluated whether the kinetics of maternal behavior could be inducted in successive experiments and found that the maternal behavior emerged gradually (significant day effect), but no difference was found between naïve hens and hens having already had a first induction experience.

On the other hand, managing the litter floor access during the oviposition time showed marked reduction of 93% in weekly percentage of floor eggs and 89% reduction in eggs per hen housed as of 76 WOA in the present study. Campbell et al. (2016a) evaluated the litter area usage in a commercial aviary (Lohmann White) and reported that the hens performed dust bathing throughout the day with peak dust bathing activity in the afternoon and late morning. This outcome is expected because in the morning period the hens are highly motivated to explore colony nests and lay their eggs; and oviposition with pre-laying activity can last 3.5 to 4.5 h after the lights come on (Hunniford et al., 2014, 2017). Therefore, dust bathing during oviposition period (early to mid-morning) is less critical due to its lower motivation priority.

Hens outside the system at night, BW and BW uniformity

The percentage of birds remaining on the litter floor declined beginning at 21 WOA. This outcome presumably arose from the birds becoming more “trained” to the lighting program (“calling” them back to the system at night) and the aviary system.

The biggest concern was with the hens in the PLA regimen because if they remained outside the system at night they could only have access to feed and water after the doors were reopened the next day. However, the percentage of hens in PLA regimen that remained on the litter area at night was quite minimal (0.010 ± 0.001% or averaging 1 per 10,000 hens), and the regimen did not affect BW or BW uniformity of the flock. Very low percentage of hens in the FLA regimen (0.040 ± 0.002%) were observed in the litter area before the lights came on. It was not clear if the presence of these hens stemmed from their natural preference or was induced by the walking sound or night light used by the caretaker when counting the hens. Campbell et al. (2016b) reported that the majority of hens in an aviary facility voluntarily returned to the system in the evening and the rest remained on the litter floor until the doors were reopened the next day.

Mortality rate

There was no evidence that managing litter access or including 1.5% experienced hens affected the flock mortality. The overall average cumulative mortality rate in the current study (14.3 ± 0.4%) was higher than the reference value for Dekalb White hens (Hendrix Genetic

Company, 2018) in alternative housing systems (94.3% livability or cumulative mortality of 5.7% by 76 WOA). Studies with aviary systems have reported cumulative mortality of 6.51-6.68% by 70 WOA (Long et al., 2016), 3.2% by 52 WOA (Sirovnik et al., 2018), 6.7-16.3% by 80 WOA (Abrahamsson and Tauson, 1995), 5-20% by 70 WOA (Nicol et al., 2006), and 11.5% by 78 WOA (Karcher et al., 2015). Therefore, the cumulative mortality rate observed in the current study by and large paralleled those of the reported field studies.

Litter conditions

The conditions of the litter were affected by managing the litter floor access, and the main reason for the impact is the extended time (approximately 6 h during light period) of litter access in the FLA regimen. Moisture content in the FLA regimen averaged 54% higher than that in the PLA regimen. Litter depth in FLA averaged 130% higher than that in PLA, which translates to the additional amount of litter removed during cleaning period. Accordingly, litter texture was different between PLA and FLA regimens in that litter in FLA was mostly caked during the first three months, hindering the hens' dust bathing activities due to lack of loose litter. The increased caking presumably arose from the thicker litter being more difficult to be dried by the ventilation air.

Litter accumulation on the floor varied with time and the litter access management (PLA vs. FLA). In this study, litter accumulation rate was higher in FLA (ranging from 0.44 to 1.15 mm/d) than in PLA (ranging from 0.22 to 0.31 mm/d). In a similar housing system, Lohmann SL White hens having 9.75 h of access to litter area per day showed a litter depth increase rate of 0.12 cm/week (0.17 mm/d) (Zhao et al., 2013). The average maximum litter depth in FLA and PLA was 6.3 cm and 2.2 cm, respectively. Campbell et al. (2016) reported that litter depth did not exceed 6.6 cm during the whole laying cycle with part-time litter access in a similar commercial aviary house.

To avoid the excessive litter accumulation in the FLA regimen it would be necessary to increase the frequency of litter removal from once every 4 months to once a month, which means the need to lock the hens in the system more often and extra labor for the cleaning. The amount of as-is litter removed averaged 130% higher (or 2.3 times) in FLA than in PLA, directly resulting from the extra time of litter floor access for the FLA regimen.

Total bacteria concentrations of litter samples were comparable to the results reported by Zhao et al. (2016) who found the bacteria concentration of litter samples (as is) in aviary system to be 9.2 ± 0.8 log CFU/g. No difference in bacteria concentration between PLA and FLA regimens was found in this study. This test was performed only at the end of the experiment, when no significant difference in litter moisture between the regimens was detected (20.6 ± 1.2% for FLA and 19.6 ± 1.2% for PLA, $P = 0.57$) and texture of the litter in both regimens was mostly loose.

Environmental conditions

There were several cold days during the experimental period; but with the supplemental heat, the indoor temperature was maintained above 20°C most of the time. The indoor temperature increased during the summer and was close to the ambient temperature, indicating that the ventilation system was well managed to remove the excess heat produced by the laying hens. The PLA or FLA regimen did not affect the microclimate. Although the hens in FLA had approximately 6 h more to spend in the litter area than the hens in PLA, no differences in temperature or RH were observed. Mixing fans located in the litter area also contributed to improving the heat distribution. The indoor RH was relatively stable, averaging 65-75% during cold season and 55-65% during warm season. The seasonal differences in RH was mostly attributed to the season-dependent ventilation rate (lower in winter and higher in summer). Similar pattern was observed by Zhao et al. (2013).

Indoor CO_2 concentration ranged from 695 ppm to 4,132 ppm (by volume), inversely related to ambient temperature. Analogous to RH, CO_2 concentration was lowest under the warmer weather and the associated maximum ventilation rate, and highest during the cold weather and the associated minimum ventilation rate.

Management of littered floor has a significant effect on ammonia concentration. Appropriate ventilation rate can reduce litter moisture content and thus ammonia release into the air (Xin et al., 2011). During the warm weather period, increased ventilation dried the litter more effectively, which reduced the ammonia generation, and further diluted its concentration. On the other hand, ammonia concentration peaked during the cold weather due to the minimum ventilation. Managing the access to litter area affected litter accumulation on the floor, moisture content, and consequently the ammonia release. Hence it was not surprising that the FLA regimen showed the highest values in all these variables (litter/manure amount, depth, moisture content, and NH_3 concentration).

Caked litter is detrimental to hens' health and welfare because a) it has higher moisture content, thus a source of higher ammonia release; and b) caked litter makes it difficult for the hens to perform dust bathing, which is one of the main purposes for providing the litter area in CF systems. On the other hand, hens in PLA deposited manure onto the manure belts in the system from 05:00 to 10:50h (lights on to system opening), while the litter area was being completely exposed (without hens), which facilitated the manure drying by the ventilation air. This process presumably reduces ammonia volatilization by reducing decomposition rate of uric acid in the manure (Sorefferle, 1965; Molloy and Tunney, 1983; Brinson et al., 1994). In addition, manure on the belts was removed much more frequently (every 3 d) than manure deposited on the litter floor (3 times per year).

During the cold weather, average ammonia concentration exceeded 25 ppm, the 8-hr exposure threshold for workers recommended by NIOSH. Ammonia concentration exceeded 25 ppm in January of 2017 in both PLA and FLA, whereas in February of 2017 and January of 2018 the exceedance occurred only in FLA. Although the month of December (2017) registered very low ambient temperature (minimum of -29˚C), the ammonia concentration data were collected on a mild day (15˚C) and proper ventilation rate was applied. Hence, the snap-shot measurement of the lower ammonia concentrations was likely not reflective of the actual levels in the cold weather.

Welfare status

This study did not find any effect of the litter access management ($P > 0.05$) on the welfare status of the laying hens by 72 WOA. However, occurrence of poor plumage condition, keel bone deformation, long claws, and foot pad dermatitis were quite frequent. We did not find statistical evidence that including 1.5% experienced hens would affect the laying hens' welfare, with the exception of the claw length ($P = 0.01$). Nonetheless, we speculate that this exception was not a cause-effect relationship, but more related to the sampling.

The overall feather coverage score of laying hens was relatively low (approximately 5 out of 14), meaning a good feather condition at 72 WOA. This outcome agreed with previous studies that evaluated welfare conditions of laying hens in conventional and alternative housing systems (Rodenburg et al., 2008; Blatchford et al., 2016). Plumage condition is associated with the hens' capability of thermoregulation, and a poor feather condition will affect the hens' welfare, increase the loss of body heat and feed energy intake to maintain homeostasis in cold weather (Sarica et al., 2008).

The incidence of keel bone deformity was moderately high, with approximately 57% of the hens showing keel bone deformation. Different studies reported frequency of keel bone deformation varying from 56% to 97% (Rodenburg et al., 2008; Käppeli et al., 2011; Wilkins et al., 2011). In CF systems, fall and collisions with perches and other parts of the housing system (Stratmann et al., 2015), and the extended perching behavior with long-term pressure on the keel bone (Tauson and Abrahamsson, 1994) are assumed to be the main causes for the high incidence of keel bone damage. Keel bone deformation or fracture has been shown to be associated with pain (Nasr et al., 2012b), decrease egg production (Nasr et al., 2012a) and elevate mortality (McCoy et al., 1996).

In this study, 35% of the hens showed some extent of foot pad disorder, which was consistent with the results revealed by Heerkens et al. (2016) that the prevalence of dermatitis ranged from 36.5% to 38.5% in ISA Brown and Dekalb White hens at 29-49 WOA. Foot pad disorders are mostly caused by prolonged pressure load on the foot pads when perching, standing on wire floor, grabbing (Weitzenbürger et al., 2006), and can be particularly painful to hens (Tauson and Abrahamsson, 1994).

Prevalence of excessive claw growth was observed in this study (78%). It can lead to easy break off, causing bleeding and possibly infection (Lay et al., 2011). Vits et al. (2005) reported that the claw length was affected by housing systems because of the different options of shortening devices used. Although not quantified, we did observe few incidences of hens with their claws stuck in the structure of the system. It can cause serious injury if the hens are not attended in a timely manner.

Conclusions

Full litter access (FLA) of the aviary housing system showed a number of shortcomings when compared with part-time litter access (PLA), including much higher incidence of floor eggs, higher ammonia concentration, more presence of caked litter, and greater accumulation of manure on the floor which necessitates more frequent removal from the barn. No difference was detected between FLA and PLA in hen welfare, mortality, BW, BW uniformity, or litter bacteria concentration. Inclusion of experienced hens (1.5%) in a young flock did not show benefit of inducing nest-laying behavior, and the young hens learned to return to the system at night quickly.

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References

Abrahamsson, P., and R. Tauson. 1995. Aviary Systems and Conventional Cages for Laying Hens: Effects on Production, Egg Quality, Health and Bird Location in Three Hybrids. Acta Agric. Scand. Sect. A — Anim. Sci. 45:191–203.

Ali, A. B. A., D. L. M. Campbell, D. M. Karcher, and J. M. Siegford. 2016. Influence of genetic strain and access to litter on spatial distribution of 4 strains of laying hens in an aviary system. Poult. Sci. 95:2489–2502.

Alm, M., H. Wall, L. Holm, A. Wichman, R. Palme, and R. Tauson. 2015. Welfare and performance in layers following temporary exclusion from the litter area on introduction to the layer facility. Poult. Sci. 94:565–573.

Blatchford, R. A., R. M. Fulton, and J. A. Mench. 2016. The utilization of the Welfare Quality ® assessment for determining laying hen condition across three housing systems. Poult. Sci. 95:154–163.

Brinson, S. E., M. L. Cabiera, and S. C. Tyson. 1994. Ammonia volatilization from surface-applied, fresh and composted poultry litter. Plant Soil 167:213–218.

Campbell, D. L. M., M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016a. Litter use by laying hens in a commercial aviary: dust bathing and piling. Poult. Sci. 95:164–175.

Campbell, D. L. M., M. M. Makagon, J. C. Swanson, and J. M. Siegford. 2016b. Laying hen movement in a commercial aviary: Enclosure to floor and back again. Poult. Sci. 95:176–187.

Chai, L., H. Xin, Y. Zhao, T. Wang, M. Soupir, and K. Liu. 2018a. Mitigating Ammonia and PM Generation of Cage-Free Henhouse Litter with Solid Additive and Liquid Spray. Trans. ASABE 61:287–294.

Chai, L., Y. Zhao, H. Xin, T. Wang, and M. L. Soupir. 2018b. Mitigating airborne bacteria generations from cage-free layer litter by spraying acidic electrolysed water. Biosyst. Eng. 170:61–71.

Colson, S., C. Arnould, and V. Michel. 2007. Motivation to dust-bathe of laying hens housed in cages and in aviaries. animal 1:433.

Cooper, J. J., and M. C. Appleby. 1995. Nesting behaviour of hens: Effects of experience on motivation. Appl. Anim. Behav. Sci. 42:283–295.

Cooper, J. J., and M. C. Appleby. 1996. Individual variation in prelaying behaviour and the incidence of floor eggs. Br. Poult. Sci. 37:245–253.

Hannah, J. F., J. L. Wilson, N. A. Cox, J. A. Cason, D. V. Bourassa, M. T. Musgrove, L. J. Richardson, L. L. Rigsby, and R. J. Buhr. 2011. Comparison of shell bacteria from unwashed and washed table eggs harvested from caged laying hens and cage-free floor-housed laying hens. Poult. Sci. 90:1586–1593.

Hansen, I. 1994. Behavioural expression of laying hens in aviaries and cages: Frequencies, time budgets and facility utilisation. Br. Poult. Sci. 35:491–508.

Heerkens, J. L. T., E. Delezie, B. Ampe, T. B. Rodenburg, and F. A. M. Tuyttens. 2016. Ramps and hybrid effects on keel bone and foot pad disorders in modified aviaries for laying hens. Poult. Sci. 95:2479–2488.

Holt, P. S., R. H. Davies, J. Dewulf, R. K. Gast, J. K. Huwe, D. R. Jones, D. Waltman, K. R. Willian, D. B. J., K. D., and G. I. A. 2011. The impact of different housing systems on egg safety and quality. Poult. Sci. 90:251–262.

Hunniford, M. E., S. Torrey, G. Bédécarrats, I. J. H. H. Duncan, and T. M. Widowski. 2014. Evidence of competition for nest sites by laying hens in large furnished cages. Appl. Anim. Behav. Sci. 161:95–104.

Hunniford, M. E., C. Woolcott, J. Siegford, and T. M. Widowski. 2017. Nesting behavior of Hy-Line hens in modified enriched colony cages. Poult. Sci. 96:1515–1523.

Jones, D. R., N. A. Cox, J. Guard, P. J. Fedorka-Cray, R. J. Buhr, R. K. Gast, Z. Abdo, L. L. Rigsby, J. R. Plumblee, D. M. Karcher, C. I. Robison, R. A. Blatchford, and M. M. Makagon. 2015. Microbiological impact of three commercial laying hen housing systems. Poult. Sci. 94:544–551.

Käppeli, S., S. G. Gebhardt-Henrich, E. Fröhlich, A. Pfulg, and M. H. Stoffel. 2011. Prevalence of keel bone deformities in Swiss laying hens. Br. Poult. Sci. 52:531–536.

Karcher, D. M., D. R. Jones, Z. Abdo, Y. Zhao, T. A. Shepherd, and H. Xin. 2015. Impact of commercial housing systems and nutrient and energy intake on laying hen performance and egg quality parameters. Poult. Sci. 94:485–501.

Kruschwitz, A., M. Zupan, T. Buchwalder, and B. Huber-Eicher. 2008. Nest preference of laying hens (Gallus gallus domesticus) and their motivation to exert themselves to gain nest access. Appl. Anim. Behav. Sci. 112:321–330.

Lay, D. C., R. M. Fulton, P. Y. Hester, D. M. Karcher, J. B. Kjaer, J. A. Mench, B. A. Mullens, R. C. Newberry, C. J. Nicol, N. P. O'Sullivan, and R. E. Porter. 2011. Hen welfare in different housing systems. Poult. Sci. 90:278–294.

Long, H., Y. Zhao, T. Wang, Z. Ning, and H. Xin. 2016. Effect of light-emitting diode vs. fluorescent lighting on laying hens in aviary hen houses: Part 1 – Operational characteristics of lights and production traits of hens. Poult. Sci. 95:1–11.

Louton, H., S. M. Bergmann, E. Rauch, C. Liebers, S. Reese, M. H. Erhard, C. Hoeborn, and A. Schwarzer. 2017. Evaluation of welfare parameters in laying hens on the basis of a Bavarian survey. Poult. Sci. 96:3199–3213.

Marino, L. 2017. Thinking chickens: a review of cognition, emotion, and behavior in the domestic chicken. Anim. Cogn. 20:127–147.

McCoy, M. ., G. A. . Reilly, and D. . Kilpatrick. 1996. Density and breaking strength of bones of mortalities among caged layers. Res. Vet. Sci. 60:185–186.

McGibbon, W. H. 1976. Floor Laying--a Heritable and Environmentally Influenced Trait of the Domestic Fowl. Poult. Sci. 55:765–771.

Mench, J. A., D. A. Sumner, and J. T. Rosen-Molina. 2011. Sustainability of egg production in the United States--the policy and market context. Poult. Sci. 90:229–240.

Molloy, S. P., and H. Tunney. 1983. A Laboratory Study of Ammonia Volatilization from Cattle and Pig Slurry. Irish J. Agric. Res. 22:37–45.

Nasr, M., J. Murrell, L. Wilkins, and C. Nicol. 2012a. The effect of keel fractures on egg-production parameters, mobility and behaviour in individual laying hens. Anim. Welf. 21:127–135.

Nasr, M. A. F., C. J. Nicol, and J. C. Murrell. 2012b. Do Laying Hens with Keel Bone Fractures Experience Pain? (B Taylor, Ed.). PLoS One 7:e42420.

Nicol, C. J., S. N. Brown, E. Glen, S. J. Pope, F. J. Short, P. D. Warriss, P. H. Zimmerman, and L. J. Wilkins. 2006. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. Br. Poult. Sci. 47:135–146.

Nicol, C. J., and S. J. Pope. 1992. Effects of social learning on the acquisition of discriminatory keypecking in hens. Bull. Psychon. Soc. 30:293–296.

Nicol, C. J., and S. J. Pope. 1994. Social learning in small flocks of laying hens. Anim. Behav. 47:1289–1296.

NIOSH (National Institute for Occupational Safety and Health). 2007. Pocket Guide to Chemical Hazards. Washington, DC.

De Reu, K., W. Messens, M. Heyndrickx, T. B. B. Rodenburg, M. Uyttendaele, and L. Herman. 2008. Bacterial contamination of table eggs and the influence of housing systems. Worlds. Poult. Sci. J. 64:5–19.

Riber, A. B. 2010. Development with age of nest box use and gregarious nesting in laying hens. Appl. Anim. Behav. Sci. 123:24–31.

Richard-Yris, M. A., and G. Leboucher. 1987. Responses to successive test of induction of maternal behaviour in hens. Behav. Processes 15:17–26.

Rodenburg, T., F. Tuyttens, K. de Reu, L. Herman, J. Zoons, and B. Sonck. 2008. Welfare assessment of laying hens in furnished cages and non-cage systems: an on-farm comparison. Anim. Welf. 17:363–373.

Sarica, M., S. Boga, and U. S. Yamak. 2008. The effects of space allowance on egg yield, egg quality and plumage condition of laying hens in battery cages. Czech J. Anim. Sci 53:346–353.

Scott, A. B., M. Singh, J.-A. Toribio, M. Hernandez-Jover, B. Barnes, K. Glass, B. Moloney, A. Lee, and P. Groves. 2017. Comparisons of management practices and farm design on Australian commercial layer and meat chicken farms: Cage, barn and free range (M Anjum, Ed.). PLoS One 12:e0188505.

Sherwin, C. M., and C. J. Nicol. 1993. Factors influencing floor-laying by hens in modified cages. Appl. Anim. Behav. Sci. 36:211–222.

Sirovnik, J., H. Würbel, and M. J. Toscano. 2018. Feeder space affects access to the feeder, aggression, and feed conversion in laying hens in an aviary system. Appl. Anim. Behav. Sci. 198:75–82.

Sorefferle, H. E. 1965. The Decomposition of Uric Acid in Built Up Poultry Litter. J. appl. Bact 28:412–420.

Stratmann, A., E. K. F. Fröhlich, A. Harlander-Matauschek, L. Schrader, M. J. Toscano, H. Würbel, and S. G. Gebhardt-Henrich. 2015. Soft Perches in an Aviary System Reduce Incidence of Keel Bone Damage in Laying Hens (C Sueur, Ed.). PLoS One 10:e0122568.

Tauson, R., and P. Abrahamsson. 1994. Foot and Skeletal Disorders in Laying Hens: Effects of Perch Design, Hybrid, Housing System and Stocking Density. Acta Agric. Scand. Sect. A - Anim. Sci. 44:110–119.

Vestergaard, K. 1982. Dust-bathing in the domestic fowl — diurnal rhythm and dust deprivation. Appl. Anim. Ethol. 8:487–495.

Vits, A., D. Weitzenburger, H. Hamann, and O. Distl. 2005. Production, egg quality, bone strength, claw length, and keel bone deformities of laying hens housed in furnished cages with different group sizes. Poult. Sci. 84:1511–1519.

Weitzenbürger, D., A. Vits, H. Hamann, M. Hewicker-Trautwein, and O. Distl. 2006. Macroscopic and histopathological alterations of foot pads of laying hens kept in small group housing systems and furnished cages. Br. Poult. Sci. 47:533–543.

Welfare Quality®. 2009. Welfare Quality® Assessment protocol for poultry. Lelystad, Netherlands.

Widowski, T. M., P. H. Hemsworth, J. L. Barnett, and J.-L. Rault. 2016. Laying hen welfare I. Social environment and space. Worlds. Poult. Sci. J. 72:333–342.

Wilkins, L. J., J. L. McKinstry, N. C. Avery, T. G. Knowles, S. N. Brown, J. Tarlton, and C. J. Nicol. 2011. Influence of housing system and design on bone strength and keel bone fractures in laying hens. Vet. Rec. 169:414.

Xin, H., R. S. Gates, A. R. Green, F. M. Mitloehner, P. A. Moore, and C. M. Wathes. 2011. Environmental impacts and sustainability of egg production systems. Poult. Sci. 90:263–77.

Zhao, Y., T. A. Shepherd, H. Li, and H. Xin. 2015. Environmental assessment of three egg production systems– Part I: Monitoring system and indoor air quality. Poult. Sci. 94:518–533.

Zhao, Y., H. Xin, T. A. Shepherd, M. D. Hayes, J. P. Stinn, and H. Li. 2013. Thermal environment, ammonia concentrations and emissions of aviary houses with white laying hens. Trans. ASABE 56:1145–1156.

Zhao, Y., D. Zhao, H. Ma, K. Liu, A. Atilgan, and H. Xin. 2016. Environmental assessment of three egg production systems – Part III: Airborne bacteria concentrations and emissions. Poult. Sci. 95:1473–1481.

Zupan, M., A. Kruschwitz, T. Buchwalder, B. Huber-Eicher, and I. Stuhec. 2008. Comparison of the Prelaying Behavior of Nest Layers and Litter Layers. Poult. Sci. 87:399–404.

CHAPTER 6. VENTILATION PERFORMANCE AND BIOENERGETICS OF DEKALB WHITE HENS IN A MODERN FULLY-OPEN AVIARY SYSTEM

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Abstract

Aviary is a cage-free housing style that allows laying hens to move freely, increasing their diurnal activities and consequently impacting their heat and moisture production rates or bioenergetics. The knowledge of bioenergetics and building ventilation rate in intensive livestock housings is important for efficient design and operation of modern animal production facilities, including proper sizing of ventilation and supplemental heat or cooling components. However, information is limited regarding ventilation performance and house-level heat and moisture production rates of laying hens in such contemporary cage-free facilities. Therefore, the objectives of this study were (i) to determine the building ventilation rate (VR) and the associated measurement uncertainty through *in-situ* calibration of ventilation fans and moniroting of fans runtime; and (ii) to quantify total heat production (THP) of the cage-free laying hens that is partitioned into house-level latent heat production (LHP) and sensible heat production (SHP) via indirect calorimetry and energy balance methods. A modern commercial aviary laying-hen building containing four hen rooms was used in this experiment, initially housing approximately 140,000 Dekalb White hens per room. The THP, SHP, and LHP were partitioned into light and dark period to evaluate the influence of hens' diurnal activities on bioenergetics. The mean VR was 4.0 ± 0.4 $m^3 h^{-1} hen^{-1}$, ranging from 0.8 to 9.1 $m^3 h^{-1} hen^{-1}$ for different seasons. Mean VR uncertainty varied with the operational static pressure (SP) and was 9.5% (10 Pa), 12.7% (25 Pa), and 17.3% (40 Pa). Overall, daily mean values were 7.5 ± 0.2 W kg^{-1} for THP, 4.8 ± 0.3 W kg^{-1} for SHP and 2.7 ± 0.2 W kg^{-1} for LHP; and THP reduced by 40% in the dark period (5.1 ± 0.3 W kg^{-1}) compared to the light period (8.5 ± 0.3 W kg^{-1}). The updated house-level heat production data are valuable to calculate VR more accurately, and improve the design and operation of ventilation systems, supplemental heating, and cooling.

Keywords: house-level heat production, moisture production, ventilation rate, sensitivity analysis

Introduction

Understanding the bioenergetics (heat and moisture production) associated with animal production systems and the interaction between animals and their environment is essential to provide an efficient design of modern animal production systems, including proper ventilation and supplemental heat and cooling (Albright, 1990; DeShazer, 2009). The design of a controlled environment with appropriate management of ventilation rates to control temperature, humidity, and noxious gases is typically based on heat and moisture production of the animals and their surroundings. The American Society of Agricultural and Biological Engineers (**ASABE**) and International Commission of Agricultural Engineering (**CIGR**) established values or guidelines on animal heat and moisture production rates (ASABE Standards, 2017; CIGR, 1999), based on studies conducted during 1950's – 1990's. Since then, many years of research has been committed to improving the understanding of animal and housing interaction. Considering that animal bioenergetics vary with genetics, nutrition, management practices (e.g., manure handling), housing equipment and design and thermal environment (Chepete, Xin, Puma, & Gates, 2004), these guidelines or “standards” are likely outdated for modern production applications.

Indirect calorimetry is a method used to calculate animal metabolic rate or total heat production (**THP)** by quantifying CO_2 production and O_2 consumption rates of the animal. Gas production and consumption are assessed by calculating room ventilation rate (**VR**) and the difference in gas concentration between fresh inlet air and exhaust air. While the environment temperature is below the temperature of the animal’s skin, VR is the main environmental factor involved in thermoregulation, and with the increase of the air velocity, the convective heat loss will be increased. VR is generally proportional to the outdoor temperature, with maximum occurring during the summer and minimum during the winter (DeShazer, 2009). THP of animals consists of sensible heat production (**SHP**) and latent heat production (**LHP**). SHP and LHP can be at the animal level (used to delineate animal thermoregulation) or at the housing level, where part of the sensible heat is converted to latent heat by evaporating moisture in the surroundings. House-level SHP and LHP data are more useful when designing ventilation system for moisture control (minimum ventilation) and temperature control (maximum ventilation). Heat and moisture production of laying hens are influenced by the indoor thermal environment (e.g., air temperature and relative humidity), affecting the partition between latent and sensible heat.

Heat production changes with hen activity (Hayes et al., 2013); and in alternative housing systems (Zhao et al., 2015), hens have access to enrichment, litter area or outdoor area contributing to the increase in hen activities. Studies on the bioenergetics of laying hens have been performed using calorimetric chambers housing conventional cages (Green & Xin, 2009a, 2009b), calorimetric chamber containing an aviary unit (Von Wachenfelt, Pedersen, &

Gustafsson, 2001), and whole-house commercial aviary (Hayes et al., 2013). However, housing systems and management practices change and vary among producers. Further, in current modern facilities, nominal hen capacities have increased from 50,000 (Hayes et al., 2013) to approximately 140,000 hens which in turn affect the ventilation design and consequently the bioenergetics.

Therefore, the objectives of this study were (i) to calculate the VR and the associated uncertainty, and (ii) to quantify THP and house-level SHP and LHP of Dekalb White laying hens in a modern commercial aviary laying-hen house. The values of THP, SHP and LHP were further partitioned into light and dark periods to evaluate the influence of hens' diurnal activities on the bioenergetics responses. CIGR and more recent data for heat and moisture production of laying hens were included for comparison purposes.

Materials and Methods

Animals and housing

This study was conducted in a newly constructed, two-story building (L x W x H of 197.5 m x 65.2 m x 7.6 m) in the Midwest US, oriented north-south, and containing four aviary (cage-free) rooms (L x W x H of 197.5 m x 30.5 m x 3.8 m). Each room contained six rows of an aviary system (Bolegg Gallery, Vencomatic, Eersel, The Netherlands) spanning the length of the room. The aviary system included three tiers of slatted floor (lower, middle, and upper tiers), two tiers of full-length curtained nests (lower and middle tiers, with average area of 86.4 cm^2/hen), nipple drinkers with drip cups (lower and middle tiers, with average of 10 hens/drinker), and two full-length chain feeders in each tier (average feeder space of 8 cm/hen). It also contained full-length galvanized steel perches (average perch space of 15.2 cm/hen), ramps at 45° from lower tier to litter area, three manure belts located below the slatted floor of each tier, a perforated manure-drying air duct, two LED tube lights (4.6 W/light) near the chain feeders and underneath the lower tier, and a scraper on the litter floor under the aviary system (Figure 6.1).

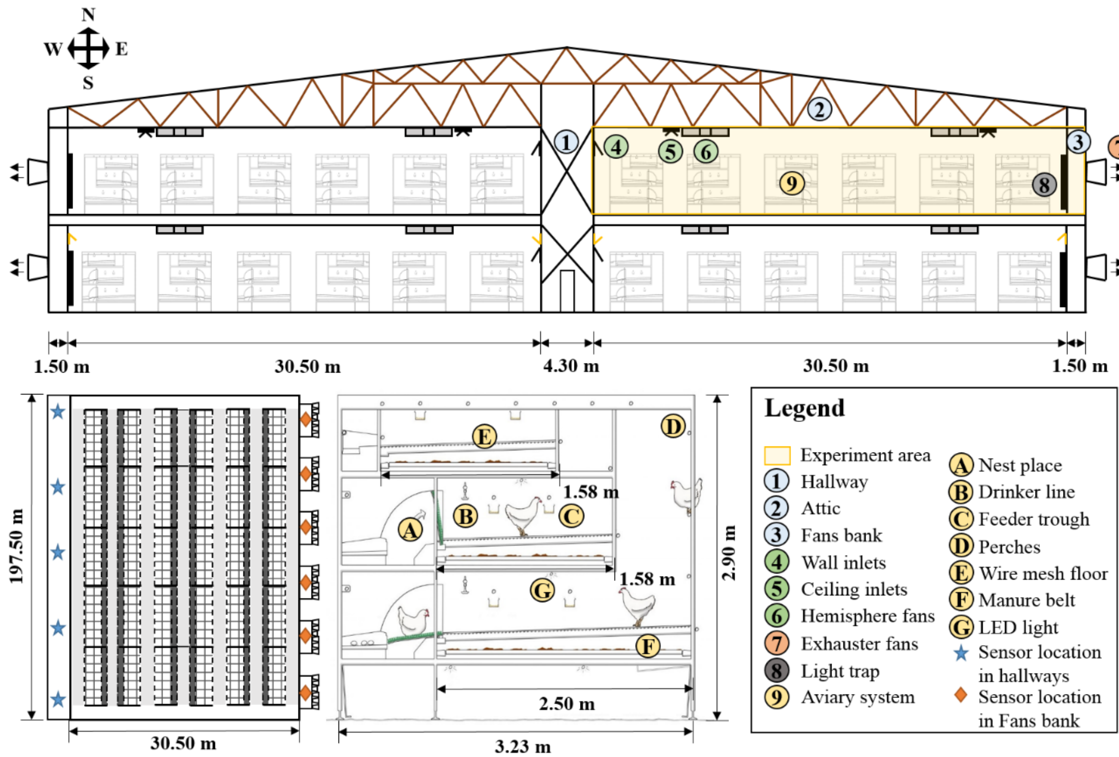

Figure 6.1 *Schematic representation of the building and the aviary system used in the experiment.*

The upper-east room (the yellow-shaded rectangle in Figure 6.1) was used in this experiment and initially housed 140,707 DeKalb White pullets at 17 weeks of age (**WoA**). Pullets were beak trimmed at the hatchery and reared in an aviary style pullet house. Light traps were installed between the bird occupied space and the exhaust fans. Light stimulation began when the house was populated. An increasing photoperiod from 12-h light and 12-h dark to 16-h light and 8-h dark was scheduled over ten weeks, following the commercial management guidelines based on hens' body weight. Commercial feed was independently distributed to each room and was provided three times a day. The metabolized energy and protein content of the feed were 13.1 ± 0.1 MJ kg-1 and 17.2%, respectively. Drinking water was provided *ad libitum*. Manure accumulated on the belts was removed from the house every three days (running one-third of the belt per day) and stored in a manure storage facility adjacent to the layer building. Litter accumulated on the floor was not removed until the end of the flock; however, a small portion was removed by the floor scraper.

Fresh air was distributed to the room by sidewall inlets located along the hallway, ceiling inlets and hemisphere fans connected to the attic. Exhaust fans provided air exchange and were located in six fans banks (Figure 6.1). More detailed description of the housing characteristics and management practices will be given in a companion paper.

Ventilation system

The mechanical ventilation system featured 36 exhaust fans: 30 large fans (130 cm diameter; VX5115F3CP; Munters Aerotech, Mason, MI, USA) and six small fans (91 cm diameter; AT365ZC; Munters Aerotech). Fans were equally distributed among six banks. Fan airflow capacity was verified by BESS Labs (tests #11369 and #93239). Fans were grouped into 13 ventilation stages. An automated system controlled the opening of the baffled inlets and the fan duty cycle according to the indoor temperature (PMSI Command III module; Poultry Management Systems Inc., Lowell, MI, USA). Fans and light traps were cleaned weekly by the farm operators.

Measurements and data collection

Temperature (°C), RH (%), and CO_2 concentration (ppm_v) were measured with 11 data loggers (±0.21°C; ±2% RH; ±50 ppm or ±5%; MX1102A, Onset Computer Corp., Bourne, MA, USA) located in the fan banks (six data loggers, one in each fans bank) and in the hallway (five data loggers equally distributed along the hallway). Room differential static pressure (**SP**) was measured with a static pressure sensor (±1.25 Pa; model 265; Setra, Boxborough, MA, USA; see locations of the sensors in Figure 6.1).

A relay controlled each of the 36 fans with relay status (on or off) monitored by the room controller (PMSI Command III module, Poultry Management Systems Inc.). Two portable Fan Assessment Numeration Systems (**FANS**; Gates et al., 2004) were used to measure *in situ* airflow for ten fans (two small fans; eight large fans). Airflow curves for the small and large fans were generated and subsequently used to calculate VR.

Room setpoint air temperature was between 21.7°C and 22.8°C during cold weather, 22.8°C and 24.4°C during mild weather, 23.9°C and 30.0°C during the warm/hot weather.

Building ventilation rate (VR)

Building VR was calculated as the sum of airflow from all operating exhaust fans (Hoff et al., 2009).

$$VR = N_S Q_S + N_L Q_L$$

where:

VR = Building ventilation rate ($m^3\ s^{-1}$)

N_S and N_T = Number of operating small and large fans, respectively.

Q_S and Q_T = Airflow rates of small and large fans, respectively ($m^3\ s^{-1}$).

The hen specific ventilation rate ($m^3\ h^{-1}\ hen^{-1}$) was calculated by dividing the VR by the number of hens in the room. The result of a measurement is always an estimate of the true value, and as a consequence, it is complete only when the variables uncertainties are presented. The uncertainty associated with VR measurement is a function of static pressure and fan airflow measurement error (Hoff et al., 2009). The combined standard uncertainty represents the standard deviation of the result. This method propagates the uncertainty via root-sum-squares (RSS) and has been used to estimate uncertainty in ventilation rates and livestock gas emission rate (Maia et al., 2015; Ramirez et al., 2014).

$$\Delta_{VR} = \sqrt{\sum_{i=1}^{N} \Delta Q_{total\ i}^{2}}$$

Where

Δ_{VR} = standard uncertainty of the building VR ($m^3\ s^{-1}$)

N = number of operating fans

ΔQ_{total} = combined uncertainty associated with fan airflow ($m^3\ s^{-1}$) for i^{th} fan

Combined uncertainty for each fan size (small and large) represents the uncertainties associated with SP measurement $\left(\frac{\partial Q}{\partial SP}\Delta SP\right)$, regression equation used to estimate airflow with a 95% confidence interval $\Delta Q_{95\%\ CI}^{2}$, and airflow measurement with the FANS unit ΔQ_{FANS}^{2}.

$$\Delta Q_{total} = \sqrt{\left(\frac{\partial Q}{\partial SP}\Delta SP\right)^{2} + \Delta Q_{95\%\ CI}^{2} + \Delta Q_{FANS}^{2}}$$

Where

$\left(\frac{\partial Q}{\partial SP}\Delta SP\right)$ = uncertainty of the differential static pressure measurement ($m^3 s^{-1}$)

$\Delta Q_{95\%\ CI}^{2}$ = uncertainty of estimating airflow rates with 95% confidence interval ($m^3 s^{-1}$)

ΔQ_{FANS}^{2} = uncertainty associated with FANS unit ($m^3 s^{-1}$)

The uncertainty associated with the SP measurement was determined by evaluating the estimated calibration equation of airflow rate related to the building operating static pressure. A second order polynomial ($Q = \beta_0 + \beta_1\ SP + \beta_2\ SP^2$) was used, and the uncertainty was described as:

$$\frac{\partial Q}{\partial SP}\Delta SP = (\beta_1 + 2\beta_2 SP)\Delta SP$$

Where

β_1 and β_2 = coefficients from the polynomial regression curve of the airflow as a function of differential static pressure ($m^3\ s^{-1}$),

SP = operational differential static pressure (Pa). Three levels of SP were considered: low (10 Pa), moderate (25 Pa) and high (40 Pa).

ΔSP = accuracy of the static pressure transducer (Pa).

The uncertainty associated with the regression equation used to estimate airflow rate with a 95% confidence interval was determined by evaluating the estimated uncertainties of the regression coefficients and the critical value related to the desired confidence interval.

$$\Delta Q^2_{95\%\ CI} = \ t_{95\%}\sqrt{\left(\ SE^2_{\beta 0} + SE^2_{\beta 1} + \ SE^2_{\beta 2}\right)}$$

Where

SE_β = Standard error of the coefficients β_0, $\beta_{1,}$ and β_2

$t_{95\%}$ = critical value obtained from t-score tables for $\alpha = 0.05$

Bioenergetics calculations

The indirect calorimetry method was used to calculate THP. Metabolic heat production of non-ruminants is related to their O_2 consumption and CO_2 production, of the following form (Brouwer, 1965; adapted by Hayes et al., 2013):

$$\text{THP} = 16.18\ O_2 + 5.02\ (CO_2 - CO_2 manure)$$

Where

THP = total heat production rate of the hens in the room (W),

O_2 = oxygen consumption rate (mL s^{-1})

CO_2= carbon dioxide production rate of the house (mL s^{-1})

CO_2 manure = carbon dioxide production rate of the manure or litter (mL s^{-1})

The ratio of CO_2 production and O_2 consumption is denoted as the respiratory quotient (RQ)

$$\mathrm{RQ} = \frac{CO_2}{O_2}$$

The CO_2 production rate was determined from the CO_2 concentration data for the inlet and exhaust air:

$$CO_2 = \mathrm{VR}\,[(CO_2)_{exh} - (CO_2)_{in}]10^{-6}$$

Where:

CO_2= carbon dioxide production rate of the house (mL s^{-1})

VR = building ventilation rate (m^3 s^{-1})

$(CO_2)_{in}$, $(CO_2)_{exh}$ = carbon dioxide concentration at the inlet and exhaust, respectively (ppm_v).

A prerequisite to calculating CO_2 production is that the CO_2 concentration in the exhaust air of the aviary house can be distinguished with sufficient accuracy from CO_2 concentration in inlet air. In practice, this means a difference in CO_2 concentration (ΔCO_2) of at least 200 ppm between the inlet and exhaust air (Van Ouwerkerk & Pedersen, 1994). Therefore, collected data where the ΔCO_2 was lower than 200 ppm_v were discarded.

The O_2 consumption rate was calculated as a function of RQ and rearranged into the expression:

$$\mathrm{THP} = 16.18\,\frac{CO_2}{RQ} + 5.02\,(CO_2 - CO_2 manure)$$

The house-level moisture production (**MP**), including the LHP of the hens and moisture evaporation from spilled water and manure, expressed as the amount of water produced in a period and was calculated from the mass-balance equation:

$$\mathrm{MP} = \rho\ \mathrm{VR}\ [(\mathrm{W})_{exh} - (\mathrm{W})_{in}]$$

Where

MP = moisture production rate of the house ($kg_{water}\ s^{-1}$)

ρ = room moist air density ($kg\ m^{-3}$)

$(W)_{in}$, $(W)_{exh}$ = humidity ratio of the inlet and exhaust air, respectively ($g_{water}\ g_{dry\ air}^{-1}$).

The air density denotes the mass per unit volume, and was calculated as (Albright, 1990):

$$\rho = \frac{1 + W}{\left(\frac{1}{P_a}\right) R_a T (1 + 1.6078\ W)}$$

Where:

ρ = air density ($kg\ m^{-3}$)

W = humidity ratio of the air ($g_{water}\ g_{dry\ air}^{-1}$).

P_a = barometric pressure of the ambient air corrected for altitude assumed to be 97,260 (Pa)

R_a = dry air gas constant, 287.055 $J\ kg^{-1}K^{-1}$

T = absolute dry bulb temperature (K)

The humidity ratio measures the mass of water vapor per unit of dry air and was calculated as:

$$W = 0.62198\left(\frac{P_w}{P_a - P_w}\right)$$

Where:

W = humidity ratio of the air ($g_{water}\ g_{dry\ air}^{-1}$).

P_w = partial vapor pressure (Pa).

P_a = barometric pressure of the ambient air corrected for altitude assumed to be 97,260 (Pa)

The partial vapor pressure is the pressure exerted by the vapor in the mixed air and was calculated as:

$$P_w = RH\ P_{ws}/100$$

Where:

P_w = partial vapor pressure (Pa).

RH = relative humidity (%).

P_{ws} = saturation vapor pressure (Pa)

The saturation vapor pressure is the pressure of the vapor when it is in equilibrium with the liquid phase, and was calculated as:

$$P_{ws} = EXP\left[\frac{C_1}{T} + C_2 + C_3T + C_4T^2 + C_5T^3 + C_6T^4 + C_7ln(T)\right]$$

Where:

P_{ws} = saturation vapor pressure (Pa)

C = For -100°C < T <= 0°C, use $C_1 = -5.6745359x10^3$; $C_2 = 6.3925247$; $C_3 = -9.677843x10^{-3}$; $C_4 = 6.22157x10^{-7}$; $C_5 = 2.0747825x10^{-9}$; $C_6 = -9.484024x10^{-13}$; $C_7 = 4.1635019$ and for -0°C < T <= 200°C, use $C_1 = -5.8002206x10^3$; $C_2 = 1.3914993$; $C_3 = -4.8640239x10^{-2}$; $C_4 = 4.1764768x10^{-5}$; $C_5 = -1.4452093x10^{-8}$; $C_6 = 0.0$; $C_7 = 6.5459673$ (ASHRAE, 2011).

T = absolute dry bulb temperature (K)

House-level LHP represents energy released from the environment as a result of the change in phase of water, in this case, vaporization, and was calculated as:

$$LHP = MP\, h_{fg}$$

Where:

LHP = house-level heat production rate of the house (W),

MP = moisture production rate of the house ($kg_{water}\ s^{-1}$)

h_{fg} = latent heat of vaporization of water, 2,427,000 J kg^{-1}.

House-level SHP represents the energy released from the environment that involves a change in the temperature and is calculated by difference as:

$$SHP = THP - LHP$$

Heat and moisture production calculations considered the body mass and population based on weekly production reports provided by the farm. The indoor temperature during the experiment period was maintained without any supplemental heat.

To assess the robustness of the indirect calorimetry-derived THP values, energy balance-based THP (THP_{bal}) was calculated from the ME intake and egg energy (EE) output, of the following form:

$$THP_{bal} = \frac{(ME - EE)10^6}{86400}$$

Sensitivity analysis

The relative importance of critical parameters on the heat and moisture production calculations was described with sensitivity analysis. The sensitivity analysis evaluated the sensitivity of the calculation of heat production (HP) to four different parameters: VR uncertainty $\left(\frac{\partial HP}{\partial VR}\right)$, CO_2 sensor accuracy $\left(\frac{\partial HP}{\partial CO_2\ sensor}\right)$, variation of RQ literature values $\left(\frac{\partial HP}{\partial RQ}\right)$, and variation of manure CO_2 production literature values $\left(\frac{\partial HP}{\partial CO_{2\ manure}}\right)$.

The RQ depends on animal metabolic rate and feed intake (Van Ouwerkerk & Pedersen, 1994) and is reflected by the feed composition, for example, RQ is 1.0 for carbohydrates, 0.8 for proteins and 0.7 for fats (DeShazer, 2009). Therefore, RQ will be lower if the animals are fed close to maintenance and increases with greater feed intake (Liu, Powers, & Harmon, 2016; Pedersen et al., 2008). The RQ of laying hens varies theoretically from 0.6 to 1.23 with averages from 0.91 to 0.95 (Chepete et al., 2004; Hayes et al., 2013; Li et al., 2005; Liu et al., 2016). This study considered an RQ of 0.95, with an increment of 10% (RQ = 1.05) for the sensitivity analysis.

The contribution of manure to total CO_2 production by laying hens has been quantified in the literature (Hayes et al., 2013; Li et al., 2005; Pedersen et al., 2008; Xin, Li, Gates, Overhults, & Earnest, 2009) and ranged from 0% to 10% of total CO_2 production. No uniform results have been found, likely because of variation in manure handling systems, stocking density, weather, litter moisture, and other uncontrolled factors. In an aviary system, the manure contribution to the total CO_2 production depended on the manure accumulation time and ranged from 4% during the summer to 8% during the summer (Hayes et al., 2013). In this study we considered a manure contribution of 4% to the total CO_2 production because of the very dry litter on the floor and frequent removal of the manure on the belts. The sensitivity analysis evaluated the impact of doubling the manure CO_2 contribution (i.e., from 4% to 8%) on the THP.

Statistical analyses

Data were tested for homoscedasticity and normality, and transformed when necessary. VR data were standardized according to the hen population and separated by photoperiod (light from 06:30 to 21:50 h; dark otherwise). Statistical analysis was performed with JMP 13.2.1 (SAS Campus Drive, Cary, NC, USA) and generalized regressions were evaluated. A p-value of 0.05 or less indicates a significant difference. Unless otherwise specified, data are presented as least squares means along with the standard error.

Results and Discussion

Environmental thermal condition

The daily average outdoor temperature ranged from 3.4°C to 28.9°C throughout the experiment period. House/room temperature ranged from 20.3°C to 30.9°C. The extreme outdoor and indoor daily mean temperatures of 28.9°C and 30.9°C, respectively, occurred once during the summer (12 of July). The next maximum temperatures were 26.4°C (outdoor) and 28.5°C (indoor). Ideally, CO_2 concentration of the fresh air is maintained at 350 ppm_v (Xin et al., 2009), and the threshold for the correct application of the CO_2 balance method is a CO_2 concentration difference between the inlet and exhaust locations (ΔCO_2) of at least 200 ppm_v (Van Ouwerkerk & Pedersen, 1994). The ΔCO_2 changed with the outdoor temperature and ranged from 202 to 2,827 ppm_v. Change in ΔCO_2 and indoor temperature concerning outdoor temperature can be observed in Figure 6.2.

As observed in Figure 6.2, during the cold weather when the outdoor temperature was ≤ 15°C, the indoor temperature was kept constant (21.0 ± 0.1°C), suggesting adequacy of the supplemental heat and adjustment of the building VR. With the mild/warm weather (outdoor temperature > 15°C), the indoor temperature increased at a rate of 0.68°C per 1°C increase in outdoor temperature. ΔCO_2 was inversely proportional to outdoor temperature and ranged from 2,827 ppm_v to 346 ppm_v when the outdoor temperature varied from 3°C to 22°C, respectively. It translates to a reduction of 131 ppm_v of ΔCO_2 per 1°C increase in that temperature interval.

When the outdoor temperature was > 22°C, ΔCO_2 was kept relatively constant at 250 ppm$_v$ ± 12 ppm$_v$.

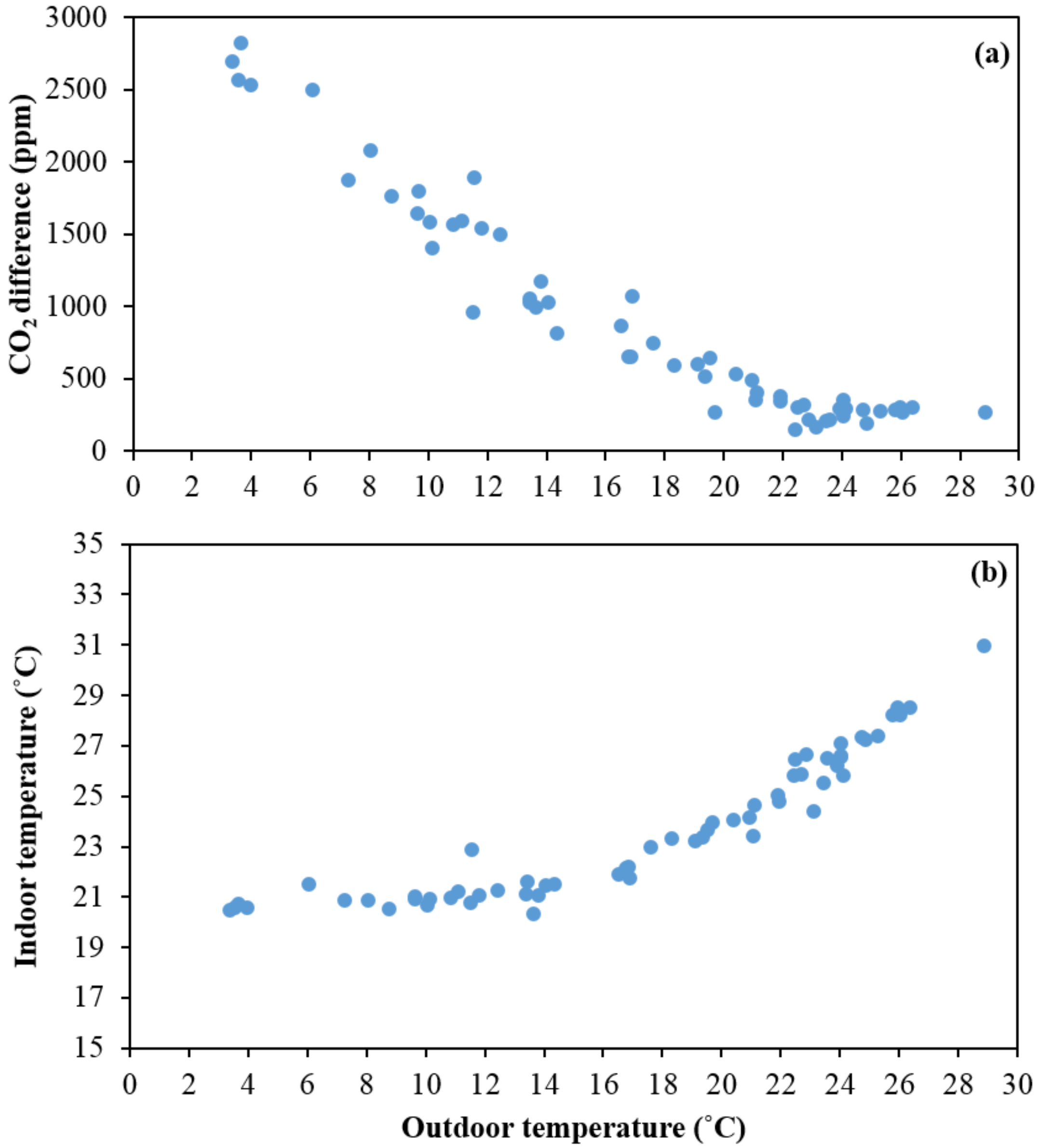

Figure 6.2 *Change of (a) CO_2 difference (ppm) and (b) indoor temperature (˚C) with outdoor temperature (˚C).*

Oliveira et al. (2019) reported the relationship between CO_2 concentration and outdoor temperature, expressed by an empirical model, where the indoor CO_2 concentration decreases with the increase of outdoor temperature. In a study to evaluate VR of a broiler house, ΔCO_2 varied from 200 ppm_v to 2,566 ppm_v corresponding to the outside temperature variation of 26°C to 16°C (Xin et al., 2009).

Ventilation rates

Theoretical and performance curves of the ten calibrated fans are shown in Figure 6.3. Compared to theoretical airflow rates (manufacturer's specification), a reduction in airflow (measured) was observed. The reduction could have been attributed to conditions of the fans (e.g., dust accumulation on the blades and shutters, tightness of the fan motor belts) and their run time (Casey et al., 2008). Airflow for the large and small fans (Q_L, Q_S) and SP followed non-linear relationships, of the following forms:

$$Q_L = -0.0021\,SP^2 + 0.0020\,SP + 11.6390\ (R^2 = 0.91, n = 58)$$

$$Q_S = -0.0021\,SP^2 + 0.0267\,SP + 4.0138\ (R^2 = 0.99, n = 12)$$

Mean VR was 4.0 ± 0.4 $m^3\ h^{-1}\ hen^{-1}$ and ranged from 0.8 to 9.1 $m^3\ h^{-1}\ hen^{-1}$. Mean VR standard uncertainty varied with operational SP and was 9.5% (SP = 10 Pa), 12.7% (SP = 25 Pa), and 17.3% (SP = 40 Pa). These uncertainties are specific for this ventilation system and building characteristics. The uncertainty of VR measurements in a commercial house of caged laying hens increased with SP, ranging from 2.3% when SP was 7.1 Pa to 12.8% when SP was 40 Pa (Rosa, Arriaga, Calvet, & Merino, 2018). The magnitude of the uncertainty values found was smaller than the values found in the current study, possibly due to the differences in calibration method and housing style. The error associated with mechanically ventilated buildings may arise from airflow profile changes during *in-situ* calibration of a given ventilation fan, and if manufacture curves are used instead of on-farm calibrated fans, the measurements can be overestimated by as much as 40% (Calvet et al., 2013).

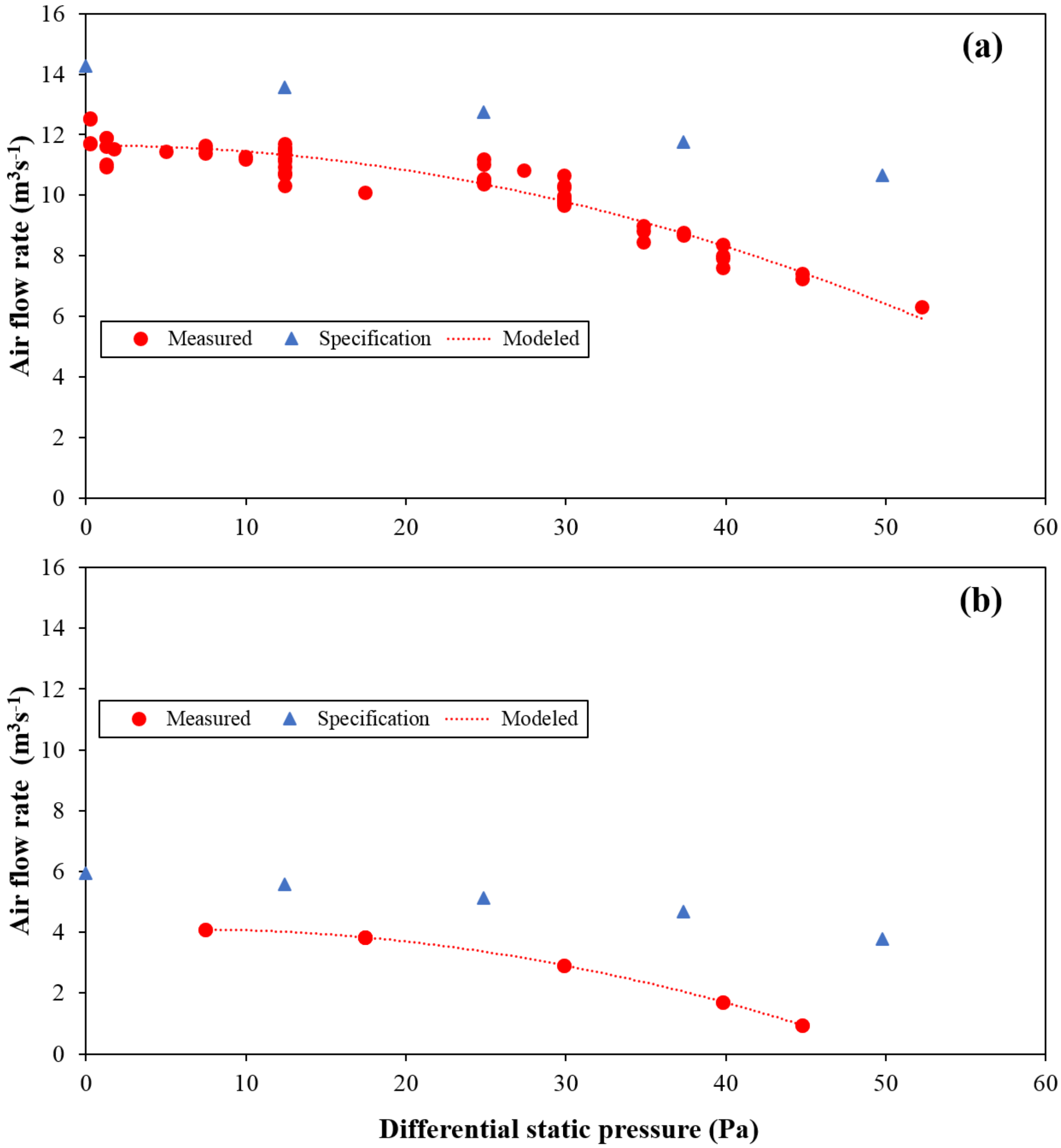

Figure 6.3 *Airflow rate curves for (a) large fans with a diameter of 130 cm and (b) small fans with a diameter of 91 cm.*

In the current study, the differences in VR from manufacture curves (specification) and the modeled values varied from 17 to 39% for the large fans, and from 27 to 55% for the small fans. As expected, higher VR was associated with warmer outdoor temperature (Figure 6.4). VR changed with outdoor temperature (T), following a non-linear trend, namely,

$$VR = 0.0181\,T^2 - 0.1506\,T + 0.9248\ (R^2 = 0.94, n = 57)$$

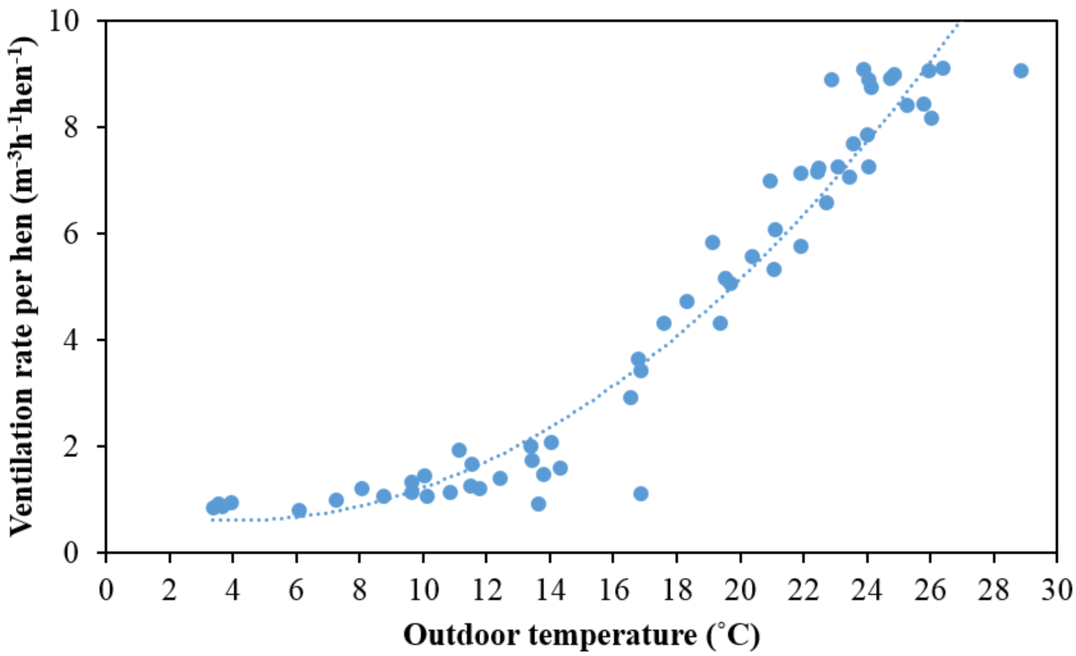

Figure 6.4 *Change of hen ventilation rate with the outdoor temperature.*

The reported VR showed differences when compared with previous studies. In manure-belt conventional cage layer houses, VR ranged from 0.4 to 5.3 $m^3\ h^{-1}\ hen^{-1}$ (Li et al., 2005), from 0.6 to 5.0 $m^3\ h^{-1}\ hen^{-1}$ (Chai et al., 2012) and from 1.1 to 11.6 $m^3\ h^{-1}\ hen^{-1}$ (Rosa et al., 2018). Alberdi et al. (2016) reported VR ranged from 0.9 to 13.3 $m^3\ h^{-1}\ hen^{-1}$ for ammonia emission quantification from enriched colony housing in Spain, and Lin et al. (2018) reported

VR from 1.9 to 8.7 $m^3 h^{-1} hen^{-1}$ when evaluating ventilation in cage-free layer houses in California. In all cases, VR was correlated with outdoor temperature.

As observed in Figure 6.5, ΔCO_2 changed with VR ($m^3 h^{-1} hen^{-1}$), following a power regression, of the following form:

$$\Delta CO_2 = 1913.6\, VR^{-0.92}\ (R^2 = 0.89, n = 57)$$

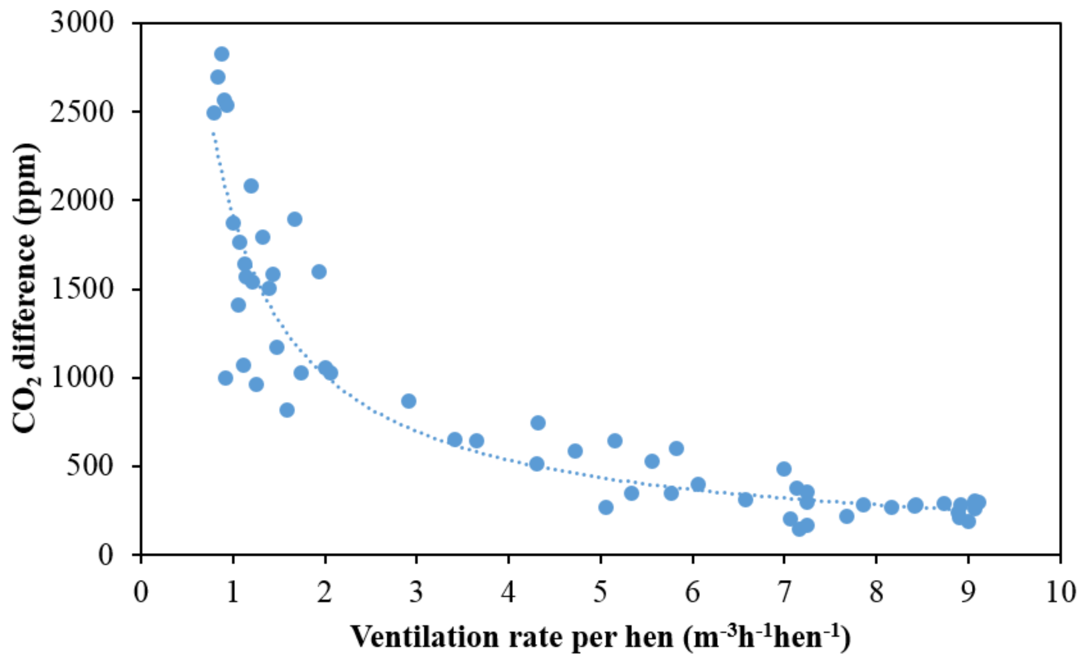

Figure 6.5. *Difference in CO_2 concentration (ppm_v) between the inlet air and the exhaust air as a function of the housing ventilation rate ($m^3h^{-1}hen^{-1}$).*

The ΔCO_2 was lowest under the maximum VR associated with the warm weather and highest under the minimum VR associated with the cold weather.

Heat and moisture production

Data from 50 days, with ΔCO_2 > 200 ppm_v, were collected from 8 July 2018 to 19 October 2018. Outdoor temperature decreased during the experiment period; however, THP did not follow an obvious trend with days (P = 0.32) or outdoor temperature (P = 0.59), suggesting that the ventilation system and the lower stocking density were effective at maintaining the indoor thermal environment within the hen's thermoneutral zone. Figure 6.6 shows THP, SHP, and LHP as a function of indoor temperature. There is no evident trend between indoor temperature and SHP (P = 0.74). However, trends between indoor temperature and LHP (P = 0.03) or THP (P = 0.03) were observed.

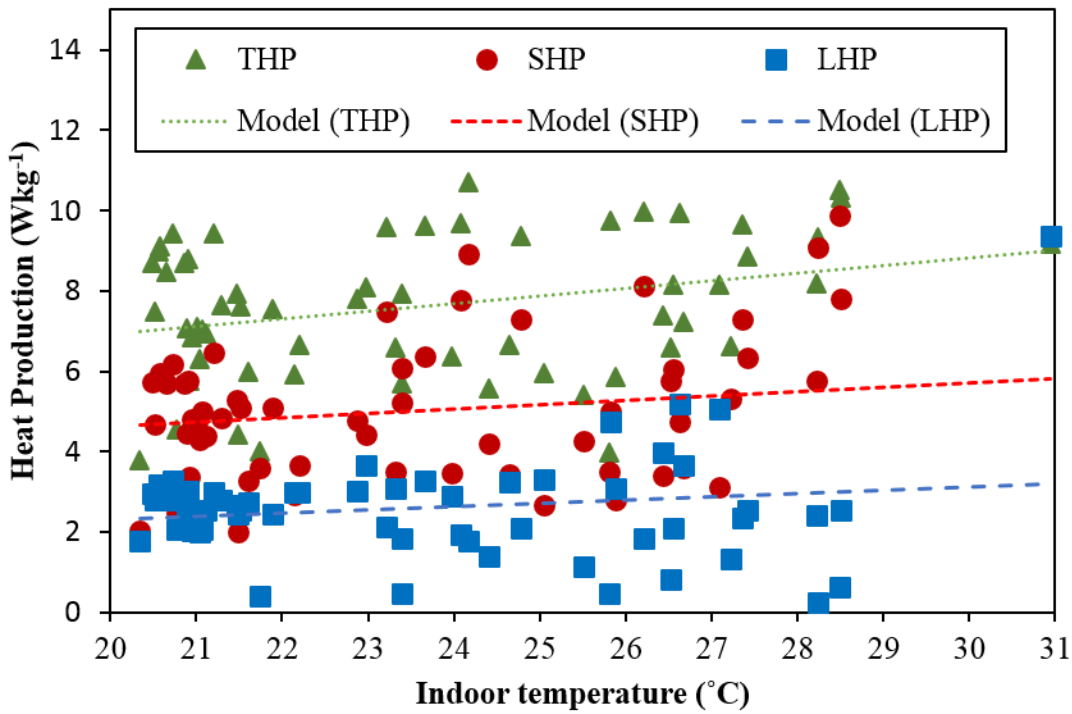

Figure 6.6 *THP and house-level LHP and SHP (W kg^{-1}) vs. indoor temperature (˚C).*

Overall, daily mean values were 7.5 ± 0.2 W kg^{-1} (THP), 4.8 ± 0.3 W kg^{-1} (SHP) and 2.7 ± 0.2 W kg^{-1} (LHP). In an experiment to evaluate the effect of stocking density and group size on heat and moisture production in conventional system inside calorimeter chambers, Green and Xin (2009a) reported that Hy-line W-36 hens (39 to 46 WOA, 1.5-1.6 kg) had THP of 6.4 to 6.6 W kg^{-1} at 24°C, 5.6 to 6.1 W kg^{-1} at 32°C and 5.9 to 6.5 W kg^{-1} at 35°C. In a cage-free aviary system, THP of Lohmann Select Leghorn layers (59 to 68 WOA, 1.6-1.8 kg) was 22% higher at 20°C than the CIGR guideline value (Von Wachenfelt et al., 2001). Hy-line Brown layers (17 to 83 WOA, 1.4-2.0 kg BW) produced 5.9 W kg^{-1} THP with 1.8 W kg^{-1} LHP in a cage-free aviary house (Hayes et al., 2013).

THP obtained from energy balance agreed well with that derived from the indirect calorimetry method. It ranged from 7.5 W kg^{-1}, when considering a 1% feed wastage, to 7.6 W kg^{-1}, when considering no feed wastage. The 1% feed wastage led to a 1.3% reduction in the energy balance-based THP.

On average, the daily LHP was about 36% of THP. This value was somewhat different from those reported by Hayes et al. (2013) (31%) and Chepete et al. (2004) (~40%). Differences in the ventilation systems, housing styles, litter conditions, bird activity level, breeds, and management practices presumably contributed to the differences observed.

A diurnal pattern of heat production was observed (Figure 6.7). THP increased as the light came on at 06:30 h (5 min of onset period) and decreased when the light went off at 21:55 h (20 min dimming period).

This pattern was previously observed by Hayes et al. (2013), suggesting the increase of HP was proportional to the activity level of laying hens in the aviary system. Overall, THP decreased by 40% in the dark period (5.1 ± 0.3 W kg^{-1}) when compared with THP during the light period (8.5 ± 0.3 W kg^{-1}) ($P<0.01$) (Figure 6.8). The reduction in THP from light to dark has been reported to be ~30% (Hayes et al., 2013), 25% (Green & Xin, 2009a), 26% (Xin et al., 1996), and 35% (MacLeod & Jewitt, 1984). The reduction of THP during the dark period in this study was higher than the values found in the literature presumably due to the higher diurnal activity level experienced by the hens in the fully open aviary system. Both SHP and LHP during the light period (5.3 ± 0.3 W kg^{-1} and 3.2 ± 0.3 W kg^{-1}, respectively) were also significantly different ($P < 0.01$) when compared with the dark period (3.5 ± 0.2 W kg^{-1} and 1.6 ± 0.1 W kg^{-1} for SHP and LHP, respectively).

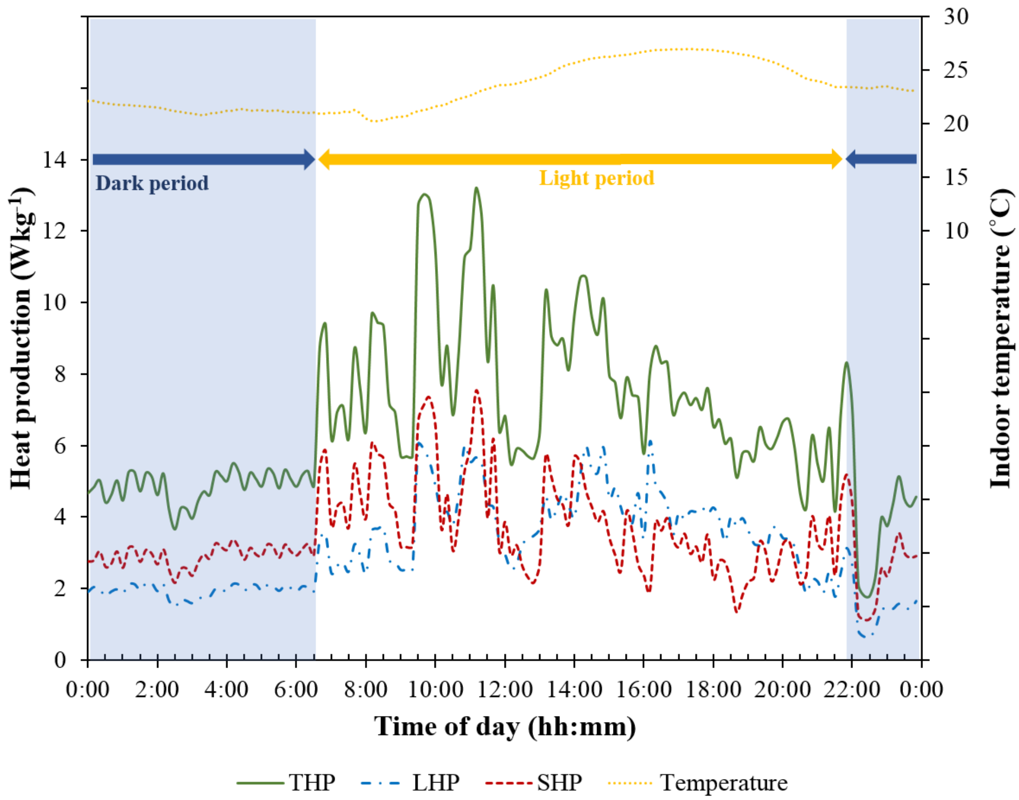

Figure 6.7 *Sample of diurnal pattern of THP, LHP, and SHP (W kg^{-1}) and indoor temperature (˚C). Lights came on at 06:30 h and went off at 21:55 h. Blue shades represent the dark period.*

The uncertainties in the measured differences in CO_2 concentrations between exhaust and inlet air also are critical and present major contributions to the uncertainties in the modeled heat production.

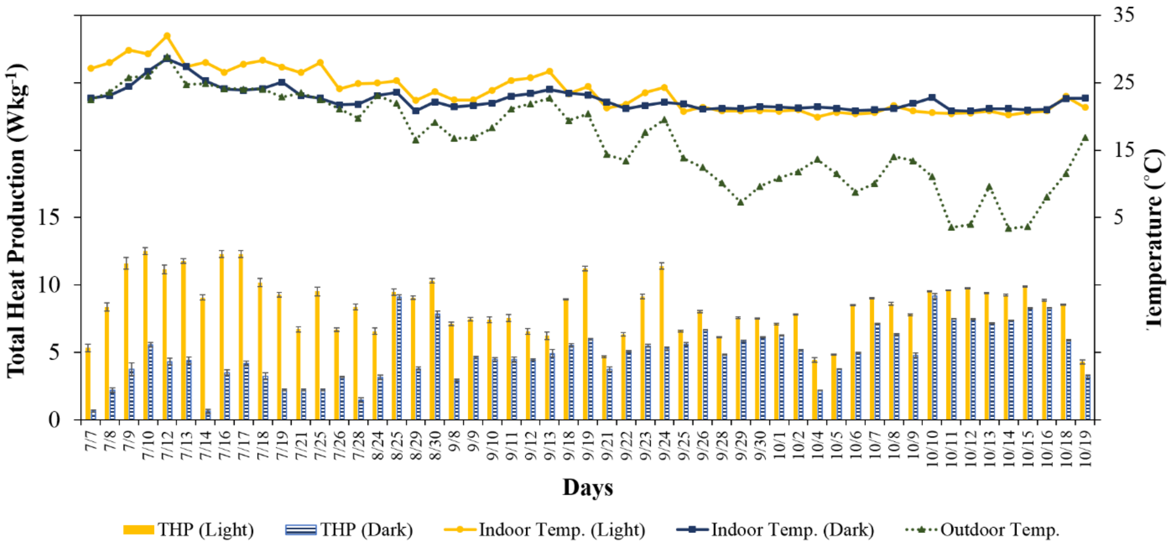

Figure 6.8 *Total heat production (W kg^{-1}) during the light and dark period as the indoor and outdoor temperature (˚C) change during the experiment period.*

Table 6.1 *Sensitivity analysis of critical parameters for their impact on the calculated total, sensible and latent heat production (THP, SHP and LHP)*

Parameter	**Base value** [1]	**Increment** [2]	**Change in heat production**		
			THP, %	**SHP%**	**LHP%**
CO_2, indoor	1,278 ppm	4% (50 ppm)	12.1	19.8	0.0
CO_2, outdoor	531 ppm	9% (50 ppm)	-12.0	-19.5	0.0
VR	4.36 m^3 h^{-1} hen^{-1}	10%	10.1	10.2	9.9
RQ	0.95	10%	-7.0	-11.4	0.0
CO_2 manure	4%	100%	-0.9	-1.4	0.0

[1] Base value is the parameter’s average or literature value used to calculate the original THP, SHP, and LHP.

[2] Increment is the parameter’s percentage increase over the base value, used to calculate the change in heat production.

As observed in Table 6.1, an increase of ~4% in indoor CO_2 concentration (50 ppm) led to approximately 12% increase in the calculated THP and 20% for SHP. On the other hand, the increase of ~9% in outdoor CO_2 concentration (50 ppm) led to a 12% decrease in the calculated THP and 20% for SHP. A 10% increment in VR led to proportional changes in the calculated THP, SHP and LHP. The sensitivity of the unmeasured parameters (RQ and CO_2 production from manure) demonstrated that the THP models are in general more sensitive to RQ, where an increment of 10% caused a ~7% reduction in THP and ~11% in SHP. A 100% increase in the assumed CO_2 production from manure (from 4% to 8%) caused a ~1% reduction in THP and SHP.

The THP found from the current study was 12% greater than that predicted by CIGR (1999; THP = $6.8m^{0.76} + 25Y_2$) for laying hens on the floor at thermoneutrality, considering the average weight of the laying hens m = 1.6 kg and Y_2 = 0.050 kg day^{-1} for consumer eggs. Therefore, the results confirmed that modern laying hens exhibit a higher metabolic rate, presumably resulting from more diurnal activities, improved nutrition and productivity.

Conclusions

This study investigated bioenergetics of Dekalb White laying hens in a modern commercial fully-open aviary housing (~140,000 laying hens capacity). In doing so, *in situ* airflow curves of ventilation fans were established and fans runtime continuously monitored to calculate building ventilation rate (VR). Carbon dioxide (CO_2) concentrations and thermal conditions of the inlet and exhaust air were continually recorded and used in quantifying the bioenergetics response. Total heat production (THP) of the hens were determined using indirect calorimetry method, and it was portioned into house-level sensible heat production

(SHP) and latent heat production (LHP). The following observations and conclusions were made.

- Daily mean hen-specific VR was 4.0 ± 0.4 $m^3h^{-1}hen^{-1}$, ranging from 0.8 to 9.1 m^3h^{-1} hen^{-1}.
- Uncertainty of VR varied with the operational static pressure (SP), i.e., 9.5% at SP of 10 Pa, 12.7% at SP of 25 Pa, and 17.3% at SP of 40 Pa.
- Daily time-weighted average (TWA) THP, LHP and SHP were 7.5 ± 0.2 Wkg^{-1}, 4.8 ± 0.3 Wkg^{-1} and 2.7 ± 0.2 Wkg^{-1}, respectively. THP decreased by 40% in the dark period (5.1 ± 0.3 Wkg^{-1}) as compared with THP during the light period (8.5 ± 0.3 Wkg^{-1}).

The house-level heat and moisture production data from this study will contribute to updating of the engineering standards or guidelines for efficient design of environmental control systems in modern cage-free layer production.

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References

Alberdi, O., Arriaga, H., Calvet, S., Estellés, F., & Merino, P. (2016). Ammonia and greenhouse gas emissions from an enriched cage laying hen facility. Biosystems Engineering, 144, 1–12. https://doi.org/10.1016/J.BIOSYSTEMSENG.2016.01.009

Albright, L. D. (1990). Environment Control for Animals and Plants. St. Joseph, Mich.: American Society of Agricultural Engineers.

ASABE Standards. (2017). Design of Ventilation Systems for Poultry and Livestock Shelters. (ASABE, Ed.), Design of ventilation systems for poultry and livestock shelters (Vol. 1986). St. Joseph, Mich.

Brouwer, E. (1965). Report of sub-committee on constant and factors. In K. L. Blaxter (Ed.), 3rd Symposium Energy Metabolism (pp. 441–443). Troon, Scotland: European Association for Animal Production.

Calvet, S., Gates, R. S., Zhang, G., Estellés, F., Ogink, N. W. M., Pedersen, S., & Berckmans, D. (2013). Measuring gas emissions from livestock buildings: A review on uncertainty analysis and error sources. Biosystems Engineering, 116, 221–231. https://doi.org/10.1016/j.biosystemseng.2012.11.004

Casey, K. D., Gates, R. S., Wheeler, E. F., Xin, H., Liang, Y., Pescatore, A. J., & Ford, M. J. (2008). On-Farm Ventilation Fan Performance Evaluations and Implications. The Journal of Applied Poultry Research, 17(2), 283–295. https://doi.org/10.3382/japr.2006-00055

Chai, L., Ni, J.-Q., Diehl, C. A., Kilic, I., Heber, A. J., Chen, Y., … Chen, L. (2012). Ventilation rates in large commercial layer hen houses with two-year continuous monitoring. British Poultry Science, 53(1), 19–31. https://doi.org/10.1080/00071668.2011.643766

Chepete, H. J., Xin, H., Puma, M. C., & Gates, R. S. (2004). Heat and moisture production of poultry and their housing systems: Pullets and layers. ASHRAE Journal, 110(2), 286–299. Retrieved from http://lib.dr.iastate.edu/abe_eng_pubs

CIGR. (1999). CIGR Handbook of Agricultural Engineering Volume II: Animal Production and Aquacultural Engineering. Retrieved from https://elibrary.asabe.org/azdez.asp?search=1&JID=6&AID=36335&CID=cig21999&T=2&urlRedirect=

DeShazer, J. A. (2009). Livestock energetics and thermal environmental management. American Society of Agricultural and Biological Engineers.

Gates, R. S., Casey, K. D., Xin, H., Wheeler, E. F., & Simmons, J. D. (2004). Fan Assessment Numeration System (FANS) Design and Calibration Specifications. Transactions of the ASAE, 47(5), 1709–1715. Retrieved from http://lib.dr.iastate.edu/abe_eng_pubs

Green, A. R., & Xin, H. (2009a). Effects of stocking density and group size on heat and moisture production of laying hens under thermoneutral and heat-challenging conditions. Transactions of the ASABE, 52(6), 2027–2032. Retrieved from http://lib.dr.iastate.edu/abe_eng_pubs

Green, A. R., & Xin, H. (2009b). Effects of Stocking Density and Group Size on Thermoregulatory Responses of Laying Hens under Heat-Challenging Conditions. Transactions of the ASABE, 52(6), 2033–2038. Retrieved from http://lib.dr.iastate.edu/abe_eng_pubs

Hayes, M. D., Xin, H., Li, H., Shepherd, T. A., Zhao, Y., & Stinn, J. P. (2013). Heat and moisture production of hy-line brown hens in aviary houses in the Midwestern U.S. Transactions of the ASABE, 56(2), 753–761. Retrieved from https://elibrary.asabe.org/azdez.asp?search=0&JID=3&AID=42663&CID=t2013&v=56&i=2&T=2

Hoff, S. J., Bundy, D. S., Nelson, M. A., Zelle, B. C., Jacobson, L. D., Heber, A. J., … Beasley, D. B. (2009). Real-Time Airflow Rate Measurements from Mechanically Ventilated Animal Buildings. Journal of the Air & Waste Management Association, 59(6), 683–694. https://doi.org/10.3155/1047-3289.59.6.683

Li, H., Xin, H., Liang, Y., Gates, R. S., Wheeler, E. F., & Heber, A. J. (2005). Comparison of direct vs. indirect ventilation rate determinations in layer barns using manure belts. Transactions of the ASAE, 48(1), 367–372. https://doi.org/10.13031/2013.17950

Lin, X., Zhang, R., Jiang, S., El-Mashad, H. M., & Xin, H. (2018). Fan and ventilation rate monitoring of cage-free layer houses in California. Transactions of the ASABE, 61(6), 1939–1950. https://doi.org/10.13031/trans.12831

Liu, Z., Powers, W., & Harmon, J. D. (2016). Estimating Ventilation Rates of Animal Houses through CO2 Balance. Transactions of the ASABE, 59(1), 321–328. https://doi.org/10.13031/trans.59.10235

MacLeod, M. G., & Jewitt, T. R. (1984). Circadian variation in the heat production rate of the domestic fowl, Gallus domesticus: Effects of limiting feeding to a single daily meal. Comparative Biochemistry and Physiology -- Part A: Physiology, 78(4), 687–690. https://doi.org/10.1016/0300-9629(84)90617-0

Maia, G. D. N., Ramirez, B. C., Green, A. R., Rodríguez, L. F., Segers, J. R., Shike, D. W., & Gates, R. S. (2015). A Novel Ruminant Emission Measurement System: Part I. Design Evaluation and Description. Transactions of the ASABE, 58(3), 749–762. https://doi.org/10.13031/trans.58.10752

Oliveira, J. L., Xin, H., Chai, L., & Millman, S. T. (2019). Effects of litter floor access and inclusion of experienced hens in aviary housing on floor eggs , litter condition , air quality , and hen welfare. Poultry Science. https://doi.org/10.3382/ps/pey525

Pedersen, S., Blanes-Vidal, V., Joergensen, H., Chwalibog, A., Haeussermann, A., Heetkamp, M. J. W., & Aarnink, A. J. A. (2008). Carbon Dioxide Production in Animal Houses: A Literature Review. Agricultural Engineering International: CIGR Journal, X. Retrieved from http://www.cigrjournal.org/index.php/Ejounral/article/view/1205

Ramirez, B. C., Maia, G. D. N., Green, A. R., Shike, D. W., Rodriguez, L. F., & Gates, R. S. (2014). Design and Validation of a Precision Orifice Meter for Ventilation Rate Control in Open-Circuit Respiration Chambers. Transactions of the ASABE, 57(6), 1865–1872. https://doi.org/10.13031/trans.57.10754

Rosa, E., Arriaga, H., Calvet, S., & Merino, P. (2018). Assessing ventilation rate measurements in a mechanically ventilated laying hen facility. Poultry Science, 98(3), 1211–1221. https://doi.org/10.3382/ps/pey524

Van Ouwerkerk, E. N. J., & Pedersen, S. (1994). Application of the carbon dioxide mass balance method to evaluate ventilation rates in livestock buildings. In XII World Congress on Agricultural Engineering (pp. 516–529). CIGR.

Von Wachenfelt, E., Pedersen, S., & Gustafsson, G. (2001). Release of heat, moisture and carbon dioxide in an aviary system for laying hens. British Poultry Science, 42(2), 171–179. https://doi.org/10.1080/00071660120048401

Xin, H., Li, H., Gates, R. S., Overhults, D. G., & Earnest, J. W. (2009). Use of CO2 Concentration Difference or CO2 Balance to Assess Ventilation Rate of Broiler Houses. Transactions of the ASABE, 52(4), 1353–1361. Retrieved from http://lib.dr.iastate.edu/abe_eng_pubs

Zhang, G., Pedersen, S., Kai, P., & Zhang, G. (2010). Uncertainty analysis of using CO2 production models by cows to determine ventilation rate in naturally ventilated buildings. In CIGR (Ed.), XVII World Congress of the International Commission of Agricultural and Biosystems Engineering. Quebec: CIGR. Retrieved from https://pure.au.dk/ws/files/44584367/CSBE100542.pdf

Zhao, Y., Shepherd, T. A., Swanson, J. C., Mench, J. A., Karcher, D. M., & Xin, H. (2015). Comparative evaluation of three egg production systems: Housing characteristics and management practices. Poultry Science, 94(3), 475–484. https://doi.org/10.3382/ps/peu077

CHAPTER 7. GENERAL CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE STUDIES

This dissertation contains a collection of scientific papers resulting from intensive laboratory experiments or extensive field monitoring studies that address some of the pressing challenges associated with alternative laying hen housing systems. Findings from these intensive studies are expected to translate into improved management practices, more efficient housing design, and enhanced productivity and welfare of the laying hens. The first three papers were developed from evaluating nesting and feeding behaviors of *individual* laying hens in a commercial enriched colony house (ECH) module. The ECH module was instrumented with a UHF RFID system, verified by a video system, which allows for automated monitoring of feeding and nesting behaviors of individual hens. The last two papers were from experiments in two styles of commercial aviary houses with the holding capacity of approximately 50,000 and 140,000 hens each house, respectively. Impacts of litter access management on hens in one of the aviary systems were evaluated; whereas house-level heat production rates of the laying hens in the fully-open cage-free housing were quantified. Listed below is a summary of the main findings and conclusions from the experiments covered in this dissertation:

1) A UHF RFID system for characterizing feeding and nesting behaviors of individual hens in an enriched colony setting has been developed and tested. The performance of the RFID system was validated by a video system. The results demonstrated that the system can be used to characterize dynamic feeding and nesting behaviors of individual poultry. The system allows for assessing impacts of housing and management factors, such as feeder space and stocking density, on feeding behaviors and feed intake of laying hens, and the number of hens feeding simultaneously. The resultant information

will contribute to the development or improvement of guidelines for housing system design and management for enhanced animal welfare and efficient use of resources.

2) Laying hens (W-36 breed) in the ECH showed similar feeding behaviors when provided a feeder space of 12.0 or 9.5 cm/hen. Albeit the presence of ample spare feeder space, as in the case of 12.0 cm/hen regimen, the hens did not exhibit the behavior of all feeding simultaneously. Hens provided with the 6.5 cm/hen feeder space spent less time at the feeder than with the 9.5 or 12.0 cm/hen feeder space and had a lower maximum number of hens feeding simultaneously than in the 12.0 cm/hen feeder space. However, no treatment effects (12, 9.5, 8.5 or 6.5 cm/hen) were observed on the hen's production performance responses of daily feed intake, water intake and rate of lay, although the number of hens involved was quite small.

3) In an ECH, 73.3 % of the W-36 laying hens used the nest box not only in the laying period but during all light period, with time spent in the nest box – TS and frequency of visits to the nest box – FV being 63.7 ± 1.4 min/hen-d and 23.4 ± 0.7 visits/hen-d, respectively, during the 16-hr light period (05:00-21:00h), as compared to 35.9 ± 0.9 min/hen-d and 10.4 ± 0.3 visits/hen-d, respectively, during the 6-hr laying period (05:00-11:00h). The number of visits per egg laid – VE was 25.7 ± 0.8 visits/egg laid in nest box during the light period and 11.4 ± 0.4 visits/egg laid in nest box during laying period. The maximum simultaneous occupancy – SO (29.0 ± 0.4%) occurred between 07:00 and 09:00 h, i.e., within 4 hours after the lights-on (05:00h). After this peak time, synchronization of hens visiting the next box decreased and remained at low levels till lights-off (21:00h). The usage of nest box during laying period differed among individual hens: 15% of the hens presented nesting pattern with few or no

random visits; 45% presented nesting pattern, with several random visits; 8.3% of the hens used the nest box intensively during the laying period, with several random visits; 21.7% of the hens had intense use of the nest box and no nesting pattern; and 10% of the hens had moderate use of nest box during laying periods and no nesting pattern. From the hens with nesting pattern: 29% visited the nest box daily at the same time; 27% visited the nest box earlier every day; 20% visited the nest box later every day; and 24% presented a mix of earlier and later visits to the nest box. Three different phases of grouped-hens nesting behavior were observed: 1) searching phase featuring exploration of nest location from 05:00 to 06:30h; 2) primary oviposition phase where the egg laying followed a linear trend with a laying rate of 0.24 ± 0.01 eggs/min in the nest box for the period of 06:30 to 09:40h; 3) delayed oviposition where the production of eggs slowed down in a non-linear manner that began at 09:40h and ended at 11:00h. Majority of the daily eggs ($95.1 \pm 0.6\%$) were laid in the nest box, while $3.8 \pm 0.9\%$ of the eggs were laid in the scratch area, and $1.1 \pm 0.6\%$ of the eggs were laid in the middle open area of the colony. Results revealed from this study help to understand and accommodate nesting behaviors of individual laying hens in ECH. They provide a set of baseline data on daily nesting behaviors of hens in a commercially practiced ECH system, which are of reference value in future system design and management.

4) Full Litter Access (FLA) of the aviary housing system showed a number of shortcomings when compared with Partial Litter Access (PLA), including much higher incidence of floor eggs, higher ammonia concentration, more presence of caked litter, and greater accumulation of manure on the floor which necessitates more frequent removal from the barn. No difference was detected between FLA and PLA in hen

welfare, mortality, body weight (BW), BW uniformity, or litter bacteria concentration. Inclusion of experienced hens (1.5%) in a young flock did not show a benefit of inducing nest-laying behavior. The young hens learned to return to the system at night quickly.

5) In a modern fully-open cage-free system located in central Iowa, with initially ~ 140,000 laying hens, the daily mean hen-specific VR was 4.0 ± 0.4 $m^3\ h^{-1}\ hen^{-1}$, ranging from 0.8 to 9.1 $m^3\ h^{-1}\ hen^{-1}$. Mean uncertainty of the VR varied with the operational static pressure (SP) and was 9.5% for SP = 10 Pa, 12.7% for SP = 25 Pa, and 17.3% when SP = 40 Pa. VR increased with the outdoor temperature, and the difference in CO_2 concentration between the inlet air and the air in the exhauster decreased with VR. Total heat production rate (THP) of Dekalb White laying hens in the cage-free housing system was quantified using indirect calorimetry method. THP was partitioned into house-level latent heat production (LHP, determined by mass balance) and house-level sensible heat production (SHP, calculated as difference between THP and house-level LHP). The daily time-weighted (TWA) mean THP, LHP and SHP were 7.5 ± 0.2 Wkg^{-1}, 4.8 ± 0.3 Wkg^{-1} and 2.7 ± 0.2 Wkg^{-1}, respectively. THP decreased by 40% in the dark period (5.1 ± 0.3 Wkg^{-1}) when compared with during the light period (8.5 ± 0.3 Wkg^{-1}). These new house-level heating production data will contribute to updating of the engineering design standards or guidelines for efficient design and operation of environmental control systems in modern cage free production.

To enhance or expand the findings of this dissertation research toward alternative laying-hen housing design, operation and management, future investigations make look into the following:

1) Lower feeder space may lead to aggression or frustration which was not quantified in the current study. It would be prudent to examine long-term impacts of feeder space on laying hen's welfare, especially for the feeder spaces that do not impair the production performance of the hen in alternative housing systems.
2) The investigation on nesting behavior was intended to provide baseline information for commercial nest design. As such, it would be beneficial to further evaluate the impact of nest size and/or configuration on hen's behavior and welfare, especially frustration and aggression as well as network association for various hen breeds.
3) The incidence of floor eggs was significantly reduced without compromising welfare of laying hens through litter access management. However, such management may still face resistance from certain retailers. Therefore, further research on system design or management practices to minimize/eliminate floor eggs in totally open cage-free systems is recommended.
4) Bioenergetics data are essential to efficient design of building ventilation systems; and they are subject to the influence of genetics, nutrition, environment, and management (e.g., manure handling). Therefore, it is advisable to expand the quantification of house-level heat and moisture production rates to include other commonly-used breeds of hens under different production scenarios.